EXHIBIT I

JAMA Oncology | Original Investigation

Analysis of Plasma Cell-Free DNA by Ultradeep Sequencing in Patients With Stages I to III Colorectal Cancer

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IMPORTANCE Novel sensitive methods for detection and monitoring of residual disease can improve postoperative risk stratification with implications for patient selection for adjuvant chemotherapy (ACT), ACT duration, intensity of radiologic surveillance, and, ultimately, outcome for patients with colorectal cancer (CRC).

OBJECTIVE To investigate the association of circulating tumor DNA (ctDNA) with recurrence using longitudinal data from ultradeep sequencing of plasma cell-free DNA in patients with CRC before and after surgery, during and after ACT, and during surveillance.

DESIGN, SETTING, AND PARTICIPANTS In this prospective, multicenter cohort study, ctDNA was quantified in the preoperative and postoperative settings of stages I to III CRC by personalized multiplex, polymerase chain reaction–based, next-generation sequencing. The study enrolled 130 patients at the surgical departments of Aarhus University Hospital, Randers Hospital, and Herning Hospital in Denmark from May 1, 2014, to January 31, 2017. Plasma samples (n = 829) were collected before surgery, postoperatively at day 30, and every third month for up to 3 years.

MAIN OUTCOMES AND MEASURES Outcomes were ctDNA measurement, clinical recurrence, and recurrence-free survival.

RESULTS A total of 130 patients with stages I to III CRC (mean [SD] age, 67.9 [10.1] years; 74 [56.9%] male) were enrolled in the study; 5 patients discontinued participation, leaving 125 patients for analysis. Preoperatively, ctDNA was detectable in 108 of 122 patients (88.5%). After definitive treatment, longitudinal ctDNA analysis identified 14 of 16 relapses (87.5%). At postoperative day 30, ctDNA-positive patients were 7 times more likely to relapse than ctDNA-negative patients (hazard ratio [HR], 7.2; 95% CI, 2.7-19.0; $P < .001$). Similarly, shortly after ACT ctDNA-positive patients were 17 times (HR, 17.5; 95% CI, 5.4-56.5; $P < .001$) more likely to relapse. All 7 patients who were ctDNA positive after ACT experienced relapse. Monitoring during and after ACT indicated that 3 of the 10 ctDNA-positive patients (30.0%) were cleared by ACT. During surveillance after definitive therapy, ctDNA-positive patients were more than 40 times more likely to experience disease recurrence than ctDNA-negative patients (HR, 43.5; 95% CI, 9.8-193.5 $P < .001$). In all multivariate analyses, ctDNA status was independently associated with relapse after adjusting for known clinicopathologic risk factors. Serial ctDNA analyses revealed disease recurrence up to 16.5 months ahead of standard-of-care radiologic imaging (mean, 8.7 months; range, 0.8-16.5 months). Actionable mutations were identified in 81.8% of the ctDNA-positive relapse samples.

CONCLUSIONS AND RELEVANCE Circulating tumor DNA analysis can potentially change the postoperative management of CRC by enabling risk stratification, ACT monitoring, and early relapse detection.

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Supplemental content

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With 1.3 million newly diagnosed cases each year, colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related deaths.[1] Despite improved surgery, implementation of screening, and advances in treatment regimens, the 5-year mortality rate for patients with CRC remains high at approximately 40%, thereby representing a significant global health burden.[2,3]

The current standard of care for patients with CRC includes surgical resection of the tumor followed by adjuvant chemotherapy (ACT) in selected patients.[4,5] Most patients with stage II CRC are not treated with ACT; however, approximately 10% to 15% have residual disease after surgery.[3] Identification of this patient population and treatment with ACT could potentially reduce their risk of recurrence. Conversely, most patients with stage III CRC receive ACT[6] despite more than 50% being cured by surgery.[7,8] Furthermore, approximately 30% of the ACT-treated patients with stage III CRC experience recurrence, making them candidates for additional therapy.[3,9] Thus, improved tools to identify the patient population who would benefit from ACT are greatly needed.

Early diagnosis of recurrent disease is another significant unmet clinical need in CRC. After completion of definitive treatment, surveillance is recommended to detect recurrence sufficiently early for potentially curative surgery.[4,5,10] Despite surveillance, many recurrence events are detected late, and only 10% to 20% of metachronous metastases are treated with curative intent.[11,12] Therefore, there is a need for better biomarkers that can detect patients at high risk of recurrence, thereby enabling appropriate follow-up and therapeutic strategies for early recurrence detection and curative treatment.[13]

Circulating tumor DNA (ctDNA) has emerged as a promising noninvasive biomarker for longitudinal assessment of a tumor throughout disease management. In CRC, there are multiple indications for which ctDNA can assist with clinical decision making.[14-18]

We report results from a prospective and observational biomarker study in patients with stages I to III CRC with an aim to demonstrate that postoperative detection of ctDNA is associated with residual disease and high relapse risk and that longitudinal analysis enables residual disease monitoring throughout the disease course. Using a personalized, tumor-specific, multiplex polymerase chain reaction (PCR)–based next-generation sequencing (NGS) method for ctDNA detection, we demonstrate that ctDNA is detected preoperatively in patients with CRC and that postoperative ctDNA analysis enables monitoring of ACT treatment effectiveness, detection of residual disease before and after ACT treatment, early detection of recurrence, and detection of actionable mutations.

Key Points

Question Does analysis of longitudinal data from circulating tumor DNA enable residual disease detection and risk-stratified postoperative management of stages I-III colorectal cancer?

Findings In this cohort study of 125 patients and 795 plasma samples from Denmark, circulating tumor DNA was associated with relapse as were current identified risk factors, both before and after adjuvant therapy and during long-term surveillance. Furthermore, longitudinal circulating tumor DNA data analysis enabled early relapse detection and assessment of adjuvant chemotherapy effectiveness.

Meaning Analysis of longitudinal data from circulating tumor DNA may have implications for postoperative management of colorectal cancer that includes guiding adjuvant chemotherapy patient selection, guiding adjuvant chemotherapy duration optimization, and enabling earlier detection of clinical relapse.

Methods

This prospective, multicenter study recruited patients with stages I to III CRC from May 1, 2014, to January 31, 2017, at the surgical departments of Aarhus University Hospital, Randers Hospital, and Herning Hospital in Denmark. Tumor tissue was collected at surgery. Blood samples (n = 829) were collected before surgery (up to 14 days preoperatively) and at postoperative day 30 (ie, sample drawn up to 14 days before or after day 30) and then at every third month until death, patient withdrawal from the study, or month 36, whichever came first. Data on postsurgery clinical intervention and other clinicopathologic information were collected for all patients (eTable 1 in the Supplement). All patients received treatment and follow-up in compliance with the national guidelines defined by the Danish Colorectal Cancer Group. The ctDNA analyses were performed retrospectively by Natera Inc, with analysts blinded to patient outcome and sample order. Neither treating clinicians nor patients were informed about the ctDNA results. Methodologic details are available in eMethods 1 to 5 in the Supplement. The study was approved by the Committees on Biomedical Research Ethics in the Central Region of Denmark and was performed in accordance with the Declaration of Helsinki.[19] All participants provided written informed consent.

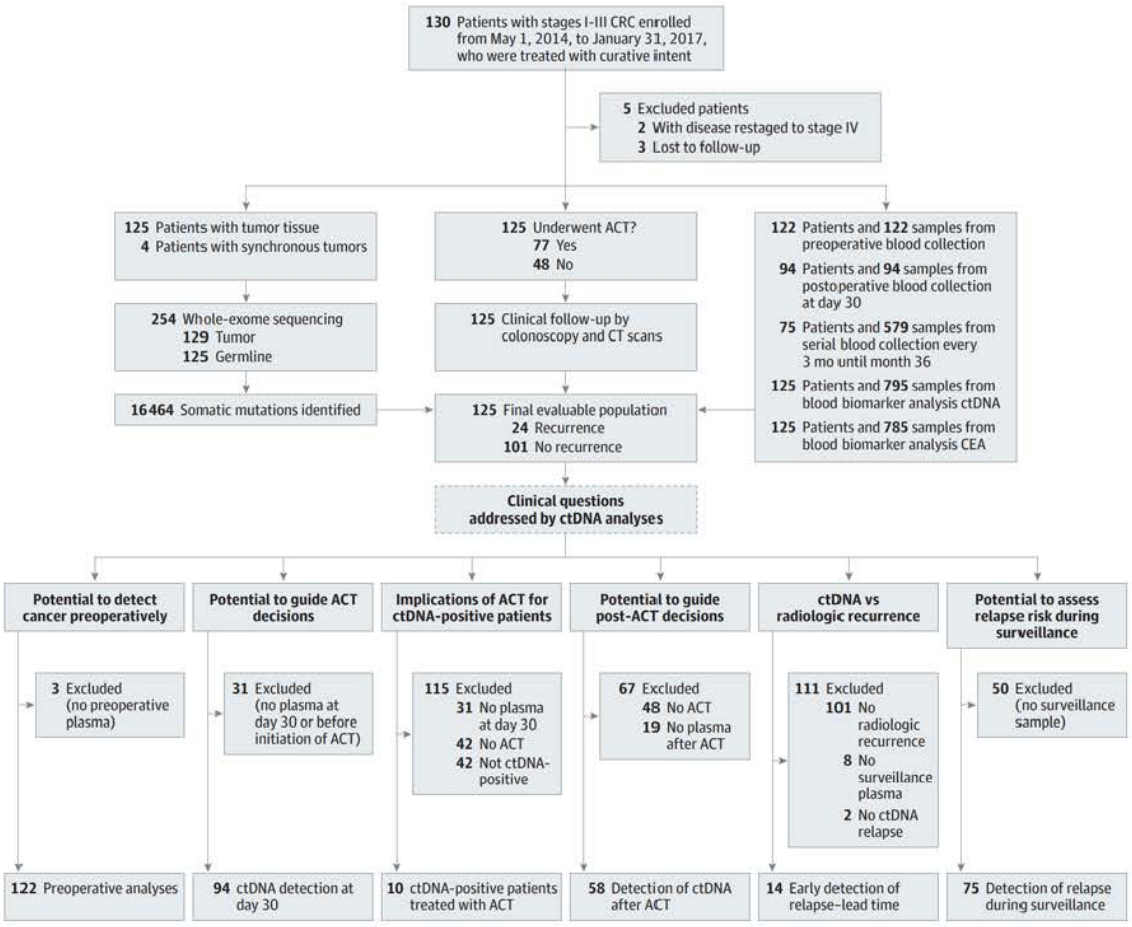
Multiplex PCR-Based NGS of Plasma Cell-Free DNA

On the basis of tumor whole-exome sequencing, 16 high-ranked patient-specific somatic single-nucleotide variants and short indels were selected for each patient. Multiplex PCR primer pairs for the chosen set of variants were generated as previously described.[20] Cell-free DNA was extracted from a median of 8.5 mL (interquartile range, 7.5-9-5 mL) of plasma. Universal libraries were created by end repair, A-tailing, and ligation with custom adapters, as previously described.[20] Next, libraries were amplified by multiplex PCR, barcoded, pooled, and sequenced on an NGS sequencing platform (HiSeq 2500 system, Illumina Inc). Plasma samples with at least 2 variants detected were defined as ctDNA positive. For details, see eMethods 2 through 9, eResults 1, and eTable 2 in the Supplement.

Statistical Analysis

The primary outcome measure was recurrence-free survival (RFS) assessed by standard radiologic criteria. Recurrence-free survival was measured from the date of surgery to the verified first radiologic recurrence (local or distant) or death as a result of CRC and was censored at last follow-up or non-CRC-related death. Patients with no follow-up were excluded from the study. Survival analysis was performed

Figure 1. Patient Enrollment, Sample Collection, and Definition of the Patient Subgroups Used to Address the Defined Clinical Questions



ACT indicates adjuvant chemotherapy; CEA, carcinoembryonic antigen; CRC, colorectal cancer; CT, computed tomography; and ctDNA, circulating tumor DNA.

using the Kaplan-Meier method. Cox proportional hazards regression analysis was used to assess the association of ctDNA and carcinoembryonic antigen (CEA) with RFS. Multivariate analysis was performed with clinical variables that were statistically significant in univariate analysis. The proportional hazards assumption was tested by a global test of the Schoenfeld residuals. All *P* values were based on 2-sided testing, and differences were considered significant at $P \leq .05$. Statistical analysis was performed using Stata IC/12.1 software (StataCorp) and R statistical software, version 2.4 for Windows (R Foundation for Statistical Computing).

Results

A total of 130 patients with International Union Against Cancer stages I to III CRC (mean [SD] age, 67.9 [10.1] years; 74 [56.9%] male) were enrolled in the study. Patient enrollment and study overview are presented in **Figure 1**. Five patients were subsequently excluded because they were lost to follow-up (n = 3) or reclassified as having stage IV disease. Patient characteristics and demographics are detailed in eTable 3 in the Supplement. Whole-exome sequencing of tumor and matched germline DNA was used to identify somatic mutations (eFigure 1 and eTable 4 in the Supplement). Tumor-specific multiplex PCR assay panels that targeted 16 mutations were designed for each patient. Ultradeep multiplex PCR-based NGS (median target coverage, >105 000 reads) (eFigure 2 in the Supplement) was used to analyze and quantify ctDNA in 795 plasma samples from 125 patients with a median follow-up of 12.5 months (range, 1.4-38.5 months) (Figure 1). Detailed information regarding ctDNA results and dynamics for all 125 patients are listed in eTable 5 and shown in eFigure 3 in the Supplement. During this period, 24 patients (19.2%) experienced radiologic recurrence (eTable 3 in the Supplement).

Preoperative Detection of ctDNA

In the 122 baseline preoperative plasma samples, ctDNA was detected in 108 of 122 samples (88.5%), with a sensitivity of 40% for stage I disease, 92% for stage II disease, and 90% for stage III disease (eFigure 4 in the Supplement). By contrast, CEA was detected in only 53 of 122 samples (43.3%) (eFigure 4 in the Supplement).

Association of ctDNA Status at Postoperative Day 30 With Risk of Recurrence

To assess whether ctDNA status is associated with residual disease and future recurrence, ctDNA analysis was performed on postoperative plasma samples. Plasma collected at day 30, before the start of ACT, was available for 94 patients. Of these patients, 84 (89.4%) were ctDNA negative, and 10 (10.6%) were positive for ctDNA (eFigure 5 in the Supplement). These ctDNA-positive patients had a significantly higher recurrence rate (70.0%, [7 of 10 patients]; 95% CI, 34.2%-93.1%) compared with those who were ctDNA negative after surgery (11.9% [10 of 84]; 95% CI, 6.3%-20.1%). The presence of ctDNA was associated with a markedly reduced RFS compared with ctDNA-negative patients (hazard ratio [HR], 7.2; 95% CI, 2.7-19.0; $P < .001$) (**Figure 2A**). In a multivariate logistic regression model, including ctDNA status and known risk factors, such as stage and lymphovascular invasion, ctDNA status was the only significant prognostic factor associated with RFS (eTable 6 in the Supplement). A subset of the patients were treated with ACT (n = 52), but even for this subset, ctDNA positivity was associated with a high risk of recurrence (HR, 7.1; 95% CI 2.2-22.0; $P < .001$) (eFigure 6 in the Supplement). The relapse rate for ctDNA-negative patients was 12%, independent of whether they were treated with ACT (5 of 42 patients) or not (5 of 42).

Association of ACT With ctDNA Clearance

Although randomized studies[21-24] have found that ACT can reduce the overall recurrence rate of stage III CRC, it is currently unknown whether ACT is specifically associated with the prevention of recurrences among the high-risk ctDNA-positive subfraction. The 10 patients who were positive for ctDNA at day 30 were all subsequently treated with ACT (Figure 2B). Of these, 7 (70.0%) relapsed, whereas 3 (30.0%) were still disease free at the end of follow-up, indicating an association between ACT and residual disease clearance in a subfraction of ctDNA-positive patients. Consistent with the association between ACT and residual disease elimination, disease-free patients with available longitudinal plasma samples had complete clearance of ctDNA during therapy and remained ctDNA negative for the duration of the study. Conversely, the 6 patients with disease recurrence who had available longitudinal plasma samples remained ctDNA positive during ACT or regained ctDNA-positive status shortly after completion of ACT.

Use of Longitudinal ctDNA Monitoring to Assess ACT Treatment Effectiveness

Longitudinally collected blood samples were available for 8 of 10 patients who were ctDNA positive before the start of ACT, which afforded us a unique opportunity to observe the changes in ctDNA levels during treatment. The ctDNA was cleared in 4 of 8 patients (50.0%) (Figure 2B), whereas in the remaining 4 patients ctDNA status remained positive throughout treatment. Strikingly, all 4 patients who did not clear ctDNA experienced disease recurrence, indicating that residual ctDNA is associated with ACT failing to eliminate the residual disease. Of the 4 patients who cleared ctDNA during treatment, 2 remained ctDNA negative in all post-ACT samples and consistently have not experienced disease recurrence, whereas the other 2 patients regained ctDNA positivity shortly after treatment and relapsed (Figure 2B).

Association of ctDNA Status After ACT With Risk of Recurrence

Because 100% of the patients who did not clear ctDNA during ACT subsequently experienced disease relapse, we hypothesized that ctDNA analysis of the first blood sample drawn after ACT can be used to identify a subgroup of patients with continued residual disease who could benefit from further treatment. Of the 58 patients with post-ACT blood samples, 7 of the 7 ctDNA-positive patients (100%; 95% CI, 59%-100%) relapsed. In comparison, of the 51 ctDNA-negative patients, 7 (13.7%) relapsed (95% CI, 6.3%-26.1%; Fisher exact test, $P < .001$). Univariate analysis showed that ctDNA status was significantly associated with recurrence (HR, 17.5; 95% CI, 5.4-56.5; $P < .001$) (Figure 2C). In a multivariate logistic regression model, including ctDNA status and risk factors, such as stage, lymphovascular invasion, and microradical resection status (eTable 7 in the Supplement), ctDNA status was the only significant factor.

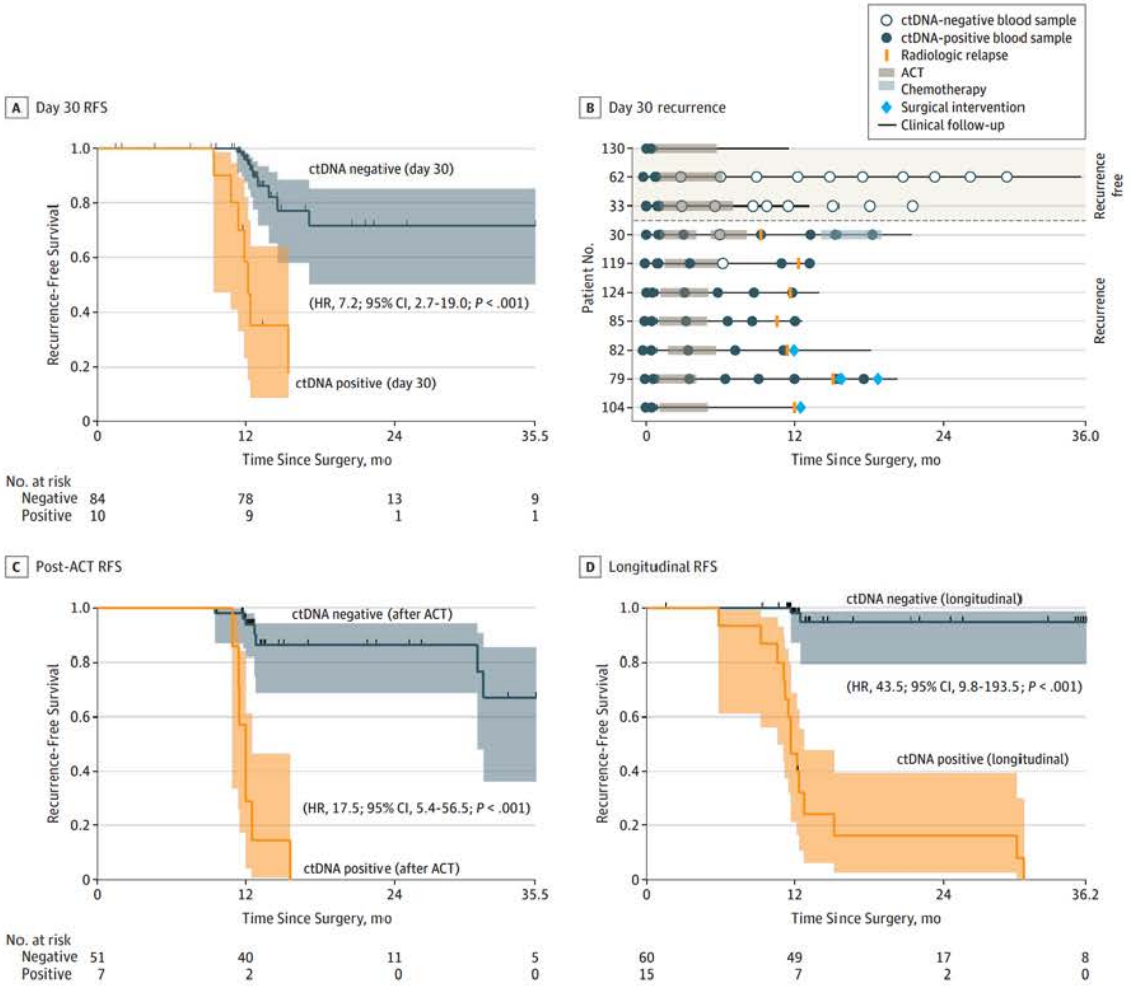
Association of Longitudinal ctDNA Analysis With Patient Outcome

Serial ctDNA analysis during surveillance after definitive treatment of the 75 patients with longitudinal collected plasma samples identified relapse with 88% sensitivity and 98% specificity. Strikingly, 14 of the 15 ctDNA-positive patients (93.3%) experienced disease recurrence compared with 2 of the 60 ctDNA-negative patients (3.3%) (Fisher exact test, $P < .001$). The ctDNA-positive patients had a markedly reduced RFS (HR, 43.5; 95% CI, 9.8-193.5 $P < .001$) (Figure 2D).

The disease course and longitudinal ctDNA results are shown in eFigure 7 in the Supplement for all 75 patients. The serial ctDNA analysis missed 2 metastatic relapses (patients 20 and 24) (eFigure 7 in the Supplement). Whole-exome sequencing of the 2 missed metastases nevertheless confirmed the presence of the mutations used for plasma screening (eTable 8 and eResults 2 in the Supplement). Longitudinal CEA analysis of this same population identified relapse with a sensitivity of 69% and specificity of 64% (eFigure 8 in the Supplement). In multivariable analysis, ctDNA was the only factor significantly associated with RFS (HR, 39.9; 95% CI, 7.5-211.0; $P < .001$) (eTable 9 in the Supplement).

The mean lead time from ctDNA detection in plasma to relapse detection by standard-of-care computed tomography was 8.7 months (range, 0.8-16.5 months) (Wilcoxon signed rank test; $P < .001$) (**Figure 3A**); by contrast, CEA revealed no lead time (eFigure 9 in the Supplement). From ctDNA detection after

Figure 2. Preoperative and Postoperative Circulating Tumor DNA (ctDNA) Monitoring in Patients With Colorectal Cancer (CRC)



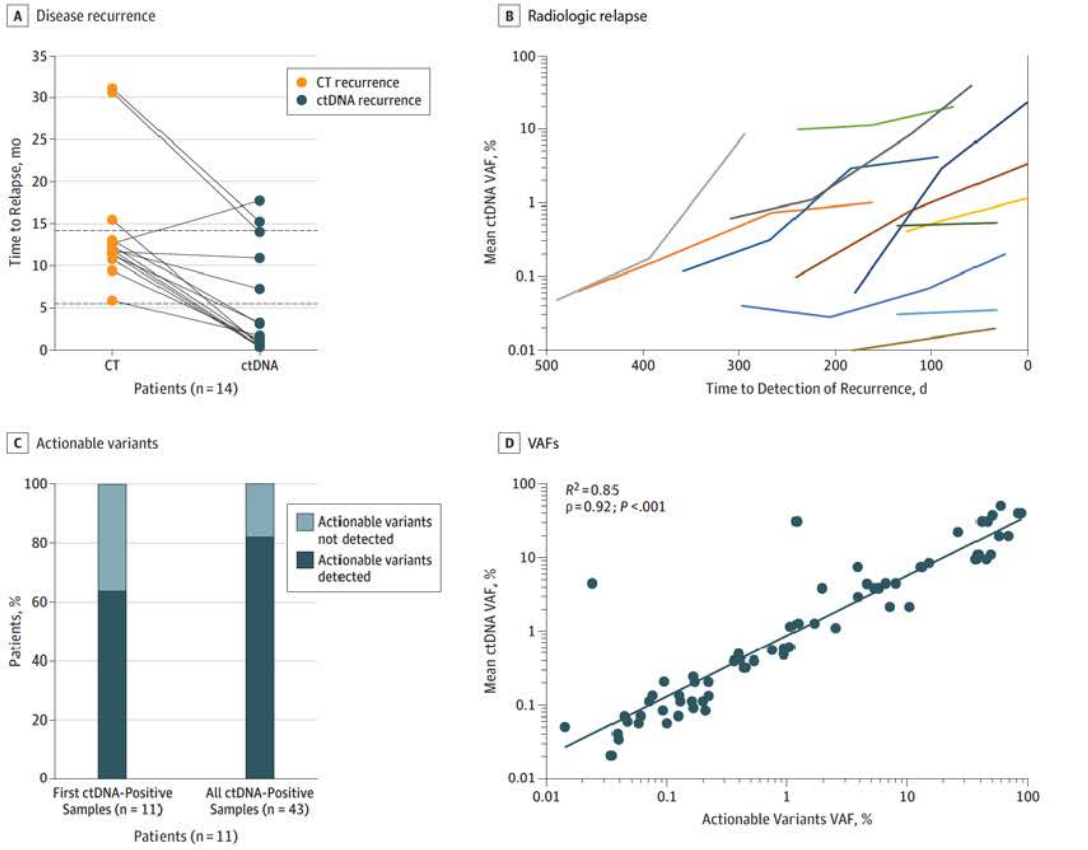
A, Kaplan-Meier estimates of recurrence-free survival (RFS) for 94 patients with stages I to III CRC stratified by postoperative day 30 ctDNA status. The 3 censored ctDNA-positive patients were all treated with adjuvant chemotherapy (ACT) and were likely cured by this treatment (see patients 33, 62, and 130 in B). B, Recurrence rate and longitudinal ctDNA status in ctDNA-positive patients receiving ACT. C, Kaplan-Meier estimates of RFS for 58 ACT-treated patients, stratified by ctDNA status at first post-ACT visit. D, Kaplan-Meier estimates of RFS for 75 patients with longitudinal samples, stratified by longitudinal post-definitive-treatment ctDNA status. A patient was classified as testing positive if 1 or more plasma samples after definitive treatment was ctDNA positive. The Kaplan-Meier plots were halted when the proportion of patients in follow-up was less than 10%. Shaded areas in the Kaplan-Meier plots indicate 95% CIs. HR indicates hazard ratio.

curative intended treatment and until radiologic relapse detection, plasma samples remained ctDNA positive. We observed an increase in the ctDNA variant allele frequency in all patients, up to 300-fold (median, 5; 95% CI, 1.4-174.0), indicating that the tumor burden often increased notably while the patients awaited radiologic detection of the relapse (Figure 3B).

ctDNA Analysis of Clinically Actionable Mutations

Having shown that longitudinal ctDNA analysis enables detection of micrometastatic disease months before radiologic relapse, we next investigated whether the ctDNA analyses in parallel could inform about the presence of potentially actionable mutations at this early recurrence time point. We identified 11 patients with disease recurrence, available longitudinal samples, and clinically actionable mutations identified by primary tumor whole-exome sequencing (eTable 10 in the Supplement). As a proof-of-concept analysis, additional multiplex PCR panels targeting the actionable mutations were designed and applied to the longitudinal samples. For 7 of the 11 patients (63.6%), an actionable mutation was detected already in the first ctDNA-positive sample; when all ctDNA-positive samples were analyzed, 9 of the 11 patients (81.8%) had actionable mutations (Figure 3C). We observed a significant correlation (Spearman $\rho = 0.92$; $R^2 = 0.85$; $P < .001$) between the

Figure 3. Association of Circulating Tumor DNA (ctDNA) Analysis With Early Detection of Relapse and Detection of Clinical Actionable Mutations



A, Comparison of time to relapse by ctDNA and standard-of-care computed tomography (CT). The mean time from surgery to relapse detection was 5.5 months (range, 0.4-17.7 months) for ctDNA and 14.2 months (range, 5.9-31.1 months for CT). Dashed lines indicate mean time in months of recurrence based on CT and ctDNA. B, For all patients with relapsing disease, the ctDNA levels in plasma increased over time from ctDNA detection to radiologic response. Early time points before and during adjuvant chemotherapy were omitted. Each colored curve represents data from a different patient. C, Fraction of recurrence in ctDNA-positive patients with actionable mutations detected in plasma. D, The actionable variants occurred with variant allele frequencies (VAFs) similar to the nonactionable variants. Association between the mean ctDNA VAF and the VAF of the actionable mutations is shown.

mean ctDNA and actionable mutation allele frequencies (Figure 3D).

Discussion

The preoperative and postoperative results presented in this study are in accordance with and expand on results presented previously.[14,15,25] We found that longitudinal ctDNA analysis in patients with stages I to III CRC can effectively detect and monitor changes in tumor burden throughout the clinical disease course. Specifically, we show that ctDNA serves as a robust biomarker for (1) postoperative and post-ACT risk stratification, (2) monitoring ACT effectiveness, (3) detection of clinical actionable mutations, and (4) early detection of recurrence. These observations have important and potential paradigm-changing implications for the future of postoperative management of CRC (eFigure 10 in the Supplement) and lay the foundation for future intervention trials to investigate the clinical benefits of ctDNA-guided management.

In the preoperative context, we found detection rates that are similar to previous studies,[25-27] confirming our ctDNA detection technique. The reliability and reproducibility of the technique were further supported by comparing the ctDNA status of the serial plasma samples and the clinical disease course. Blood samples drawn after curative treatment were expected to test negative for ctDNA. Consistent with this theory, 455 of the 456 postoperative serial blood samples (99.8%) from patients without disease relapse were ctDNA negative (eFigure 7 in the Supplement). By contrast, the serial analysis of the 16 patients with disease relapse detected ctDNA in 14 patients (87.5%) (eFigure 7 in the Supplement). Furthermore, the serial samples were persistently positive. Only in cases of clinical intervention did the ctDNA status change from positive to negative (eg, patients 75 and 119) (eFigure 7 in the Supplement).

Currently, decision making for ACT treatment is based on risk stratification by stage and clinical risk factors. We found that in multivariate analysis ctDNA status (among stage, CEA, and other high-risk factors) was the only significant factor associated with recurrence. This suggests that ctDNA analysis may be a better tool for identifying high risk patients. Hence, in the future, it may be possible to use ctDNA-analyses to identify a ctDNA positive subgroup of patients with stages I and II disease who could potentially benefit from ACT (eFigure 10 in the Supplement, trial 1). We and others are currently conducting trials to assess the clinical benefit of ctDNA-based patient selection in this setting (eg, IMPROVE-IT [Intervention Trial Implementing Noninvasive Circulating Tumor DNA Analysis to Optimize the Operative and Postoperative Treatment for Patients With Colorectal Cancer][28] and Circulating Tumour DNA [ctDNA] Analysis Informing Adjuvant Chemotherapy in Stage II Colon Cancer[29]). We also found that ctDNA-negative patients have similar low risk of relapsing, independent of whether or not ACT was administered. Hence, in the future, it may be possible to withhold ACT from ctDNA-negative but clinically high-risk patients (those with stage III disease), with a minimal alteration in their relapse risk (eFigure 10 in the Supplement). This patient group could be offered active ctDNA-based surveillance instead of ACT, thus sparing the many patients who are cured by surgery alone from the toxic effects of chemotherapy. In addition, in the post-ACT setting, where there are no current risk markers, we demonstrate that ctDNA analysis identifies patients who still have residual disease. This population may benefit from intensified therapeutic treatment.

We also found that longitudinal ctDNA monitoring before, during, and after ACT provides a patient-level measurement of ACT effectiveness. The 30% of patients who cleared ctDNA and remained negative in all subsequent samples stayed disease free throughout the study. Thus, our study provides first-line evidence that ACT can reduce the risk of recurrence in ctDNA-positive patients. This risk reduction is similar to that estimated when ACT is given to all patients with stage III colon cancers.[21-24] We found that all patients who did not clear ctDNA had disease relapse within a year of completion of ACT. In addition, all patients with only transient clearance of ctDNA also experienced relapse. These findings need to be further validated with larger studies. Future clinical trials that incorporate ctDNA clearance in the study design may allow for patient-level, real-time measurement of therapy effectiveness.

In the postoperative context, ctDNA monitoring showed a significant improvement in relapse detection compared with standard-of-care radiologic imaging, demonstrating a significant lead time of 8.7 months ($P < .001$). Of importance, while patients were awaiting radiologic detection, their ctDNA levels increased 5-fold, indicating that tumor burden increases markedly during the 8.7 months of lead time. Current guidelines recommend surveillance after curative CRC surgery.[4,5,10] Nevertheless, most relapse events are detected too late to be eligible for curative intervention.[11] The early detection of residual disease by ctDNA analysis may provide an opportunity for earlier radiologic detection (eFigure 10 in the Supplement); potentially, ctDNA status can be used to guide the frequency of radiologic imaging, the optimal scheduling for which is still debated.[12,30] In addition to detecting residual disease months before radiologic relapse, we also found that ctDNA could inform about the presence of potentially actionable mutations. In the future, ctDNA analysis may allow earlier implementation of targeted therapies in the recurrence setting.

Limitations

There are potential limitations to our study, including the modest sample size of patients with recurrent CRC and the analysis of multiple patient subsets. In any case, the consistency of the results in the serial analyses documents the robustness, reproducibility, and reliability of the reported findings.

Conclusions

Our results suggest many potentially paradigm-changing clinical applications of ctDNA in CRC and provide a framework for future clinical trials to investigate the clinical benefits of ctDNA-guided disease management.

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Correction: This article was corrected on June 14, 2019, to fix incorrect data in the Abstract and the caption of Figure 3.

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Acquisition, analysis, or interpretation of data: Reinert, Henriksen, Christensen, Sharma, Salari, Sethi, Knudsen, Nordentoft, Wu, Tin, Heilskov Rasmussen, Vang, Shchegrova, Frydendahl Boll Johansen, Srinivasan, Assaf, Balcioglu, Olson, Dashner, Hafez, Goel, Rabinowitz, Billings, Sigurjonsson, Dyrskjøt, Swenerton, Aleshin, Husted Madsen, Stribolt, Palmelund Krag, Iversen, Gotschalck Sunesen, Lin, Zimmermann, Lindbjerg Andersen.
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Supervision: Reinert, Henriksen, Sharma, Sethi, Tin, Billings, Sigurjonsson, Swenerton, Aleshin, Iversen, Lin, Zimmermann, Lindbjerg Andersen.

Conflict of Interest Disclosures: Drs Sharma, Salari, Wu, Tin, Shchegrova, Assaf, Balcioglu, Hafez, Goel, Rabinowitz, Billings, Swenerton, Aleshin, Lin, and Zimmermann, Messrs Sethi, Srinivasan, Olson, and Dashner, and Ms Navarro, reported receiving support from Natera Inc outside the submitted work. Dr Billings reported receiving support from Trovagene, OmniSeq, MissionBio, and Metastat outside the submitted work. Dr Zimmermann has a pending patent for Provisional. Dr Lindbjerg Andersen reported receiving grants from Novo Nordisk Foundation, Danish Council for Strategic Research, Danish Council for Independent Research, and Danish Cancer Society during the conduct of the study. No other disclosures were reported.

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Supplementary Online Content

Reinert T, Henriksen TV, Christensen E, et al. Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. *JAMA Oncol.* Published online May 9, 2019. doi:10.1001/jamaoncol.2019.0528

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods 1. Carcinoembryonic Antigen (CEA) Analysis
CEA analysis was performed on a Cobas e601 platform (Roche), according to the manufacturer's recommendations using 500 µL serum. The threshold levels were set to 4.0 µg/L and 6.0 µg/L for non-smokers and smokers, respectively, as recommended by the analysing hospital. A person who had not smoked for 8 weeks before sample collection was considered a former smoker.

eMethods 2. Sample Collection and DNA Extractions
Tumor tissue was collected from all patients, either as fresh frozen (n=102) or as formalin fixed and paraffin embedded tissue (FFPE) (n=27). Four patients presented with synchronous CRCs; from these patients, tissues from both tumors were collected. From three patients with relapse, metastatic tissue was also collected. Constitutional DNA matching all patients was extracted from peripheral blood leukocytes.
Primary fresh frozen or formalin fixed paraffin embedded (FFPE) tissue samples were estimated to have a median pathological tumor cellularity of 50% (range 20-90%). DNA was extracted using the Puregene DNA purification kit (Gentra Systems) or using the QiAamp DNA FFPE tissue kit (Qiagen).

eMethods 3. Whole Exome Sequencing (WES)
WES was performed by the Department of Molecular Medicine on matched tumor DNA (derived from primary fresh frozen and FFPE tissue) and buffy coat DNA (eTable 4) as previously described [1]. Libraries of tumor and matching germline DNA were prepared using 100-500 ng DNA and captured by SeqCapEZ MedExomePlusV1_hg19 panel (Roche) with a total (primary and capture) target size of 72 megabases. The MedExomePlusV1_hg19 panel was customized with 1042 SNP sites located throughout the genome. FastQ files were prepared using bcl2fastq2 (v2.20.0.422) and quality checked using FastQC (v0.11.5). Adapters were removed using Trim Galore! (v 0.4.1) The trimmed tumor and germline samples were treated according to the GATK (v3.7) best practices. Reads were mapped to the hg19 reference genome using bwa-mem (v0.7.12) and PCR duplicates were marked for filtering in the downstream analysis using Picard MarkDuplicates (v2.0.1). Local realignment around Indels and recalibration of base quality scores were then performed using GATK IndelRealigner and GATK BaseRecalibrator, respectively. As a quality control for all samples captured by the MedExomePlus panel, tumor and germline alignments were checked using allele counts for 1042 ID SNP sites. Briefly, genotype analysis of the 1042 fingerprint SNP sites served as a control for DNA contamination or sample and/or barcode mix-ups. The analysis was performed as previously described.[2] Samples were flagged and eliminated in situations where the average minor allele frequency at homozygous sites in a patient-matched normal was observed to be >1%. Samples with more than 55% heterozygous SNP sites were eliminated as such percentages indicate large-scale contamination of DNA from another individual. [2] SNVs and Indels were called using MuTect2 with information from COSMIC (v84) and dbSNP (v138). Default settings were applied, however, the threshold for maximum alternate alleles in the germline was raised. A custom filter selecting variants only vastly more present in the tumor and in regions with low noise, was subsequently applied as described earlier [3]. Furthermore, variants identified, but filtered, using MuTect2 were rescued if identified with high confidence using VarScan2 (2.4.1). All variants passing the applied filters were subjected to analysis on the activity of mutational signatures. Variants were initially loaded into a VRanges object and the sequence context was subsequently extracted using the SomaticSignatures R package [4,5]. De novo extraction of mutation signatures was not applied due to the size of the cohort. The mutational profiles identified in our samples were instead projected onto the known COSMIC signatures using the MutationalPatterns R package [6,7]. We identified signatures 1, 6 and 10, as previously reported for CRC[8], but also a strong activity of signature 15 and 18 in a subset of samples.

eMethods 4. Blood Collection and Plasma Isolation
Blood samples were collected in K2-EDTA 10 ml tubes (Becton Dickinson) at Aarhus University Hospital. All samples were processed within 2 hours of collection by double centrifugation of the blood at RT, first for 10 minutes at 3000g, followed by centrifugation of plasma for 10 minutes at 3000g. Plasma was aliquoted into 5 mL cryotubes and stored at −80°C.

eMethods 5. Cell-Free DNA Extraction and Quantification
Up to 10 ml of plasma per case was used for this study (range, 2–10 mL; median 8.5 mL) and cfDNA was extracted using the QIAamp Circulating Nucleic Acid kit (Qiagen) and eluted into 50 µL DNA Suspension Buffer (Sigma). Each cfDNA sample was quantified by Quant-iT High Sensitivity dsDNA Assay Kit (Invitrogen). In 125 patients, cfDNA was isolated from a total of 795 serial plasma samples.

eMethods 6. Plasma DNA Libraries and Plasma Multiplex-PCR NGS Workflow

Up to 66 ng (20,000 genome equivalents) of cfDNA from each plasma sample was used as input into library preparation. The cfDNA was end-repaired, A-tailed, and ligated with custom adapters, as previously described [9]. The purified ligation product was amplified (20 cycles) and purified using Ampure XP beads (Agencourt/Beckman Coulter). Patient-specific somatic variants were identified by analyses of primary tumour and matched normal WES samples for all patients. Clonality of variants was inferred based on the estimated proportion of cancer cells harboring the variant as described in McGranahan et al [10]. Note that clonality inference from samples with low tumor cell fraction is limited due to a fairly flat distribution of variant allele frequency. Observed VAF in tissue and sequence context of variants were used to prioritize somatic SNVs and short INDELs identified for each tumour expected to have a low plasma background mutation rate. The Signatera amplicon design pipeline was used to generate PCR primer pairs for the given set of variants. Variant prioritization takes into account several factors such as clonality, allele fractions, and SNV type. Design of the 16-plex PCR assays is based on Natera's optimized set of parameters which includes selecting non-interacting set of primer-pairs. Each variant gets its own PCR primers and amplicon in order to ensure targets aren't sharing reads. For each patient, 16 highly ranked compatible amplicons were selected for the custom patient-specific panel. The PCR primers were ordered from Integrated DNA Technologies. An aliquot of each library was used as input into the associated patient-specific 16-plex PCR reaction. Samples were amplified using the patient-specific assay and barcoded, followed by product pooling. Sequencing was performed on an Illumina HiSeq 2500 Rapid Run with 50 cycles of paired-end reads using the Illumina Paired End v2 kit with an average read depth of >105,000X per amplicon. All paired-end reads were merged using Pear software[11]. Bases that did not match in forward and reverse reads or that had a low quality score were filtered out to minimize sequencing errors. Merged reads were mapped to the hg19 reference genome with Novoalign version 2.3.4 (http://www.novocraft.com/). Amplicons with less than 5000x sequencing coverage were excluded from analyses and samples with less than 8 passing amplicons failed sequencing coverage QC.

eMethods 7. Plasma Variant Calling

A large set of negative control samples (~1000) were pre-processed to build a background error model. For each target variant using mutant and reference alleles depth of read, a confidence score was calculated on the basis of the error model, as described in Abbosh et al. 2017 [9]. As previously, a plasma sample with at least 2 variants with a confidence score above a predefined algorithm threshold was defined as ctDNA positive [8]. Signatera technology allows confident detection down to 0.03% VAF and lower VAF levels are also observable in many cases [12].

eMethods 8. Quality Control and Signatera RUO Workflow

For the Signatera RUO plasma workflow, a strictly-controlled semi-automated lab process was performed by trained personnel with signed-off SOPs and witnessing. The process, reagent, and equipment information were captured electronically and uploaded to a database with built-in integrity checks. A total out of 795 plasma samples 793 (99%) passed our sample QC process. In order to track sample integrity, SNP tracers (45 frequent SNPs) were used to measure concordance between a patient's samples. A genotyping concordance score was calculated for all plasma samples by comparing to their matched normal tissue genotypes. Samples were considered to be from different patients if less than 85% of their SNPs showed identical genotype calls. The genotype calls are produced by Natera proprietary algorithm that fits a distribution to observed reads, calculates the probabilities of a particular genotype, and assigns the highest probability genotype to a SNP. A total of 421 sequencing samples including all plasma samples from recurrence patients were tested by SNP tracer to check the concordance between the plasma sample and its corresponding tissue biopsy. All but one plasma sample passed the concordance QC (eFigure 2).

eMethods 9. Time Requirement

The turnaround time for Signatera is broken down into the first blood collection timepoint and all subsequent blood collection timepoints. The first blood collection timepoint entails the following steps: a) tumor tissue and whole blood-based personalized assay design and b) plasma processing, ctDNA analysis, and reporting. The turnaround time for timepoint 1 is less than 4 weeks. All subsequent blood collection timepoints involve only the plasma processing, ctDNA analysis, and reporting, the turnaround time for which is 1-2 weeks. Detailed steps for each of the timepoints are listed below.

Timepoint 1

The clinician will be provided with a blood collection kit (composed of two Streck blood tubes and an EDTA tube) simultaneously with a tissue collection kit. The tissue collection kit and EDTA tube will be used in Step 1. Plasma from the two Streck tubes gets isolated and cfDNA is extracted. cfDNA gets used in Step 2.

Step 1
The clinician is expected to send in the patient's tumor block or FFPE slides from surgery/biopsy along with their blood tubes. It takes approximately 2.5 weeks from the time of tissue block or slides receipt for the following steps: tumor tissue block or slide pathology (sectioning, slide processing), DNA extraction from both tumor FFPE slides and matching whole blood, whole exome sequencing (WES) of tumor and matched normal DNA, WES analysis, variant selection and personalized assay design, primer ordering, primer receipt, and primer pooling. This patient-specific Signatera primer pool/assay is stored and gets accessioned when subsequent blood samples from the same patient are received for ctDNA testing.

Step 2
As soon as the personalized assay has been designed in step 1, the cfDNA can be made into a universal adapter library. An aliquot of the library is combined with the patient-specific multiplex primer pool for the mPCR assay where only the targets of interest are amplified. The mPCR amplicon product is barcoded, pooled, and sequenced. Sequencing data goes through QC followed by the ctDNA calling algorithm. The report is then issued.

Subsequent blood collection timepoints
For all subsequent blood collection timepoints, the clinician is sent only the blood collection kit with the 2 Streck tubes. Plasma from the two Streck tubes gets isolated and cfDNA extracted. This cfDNA then gets used as described above in Step 2 with the patient-specific Signatera assay (originally designed in Step 1)."

eResults 1. Performance Estimates of the Assay Based on Number of Mutations for Assay Design
To demonstrate the effect of the number of mutations tracked by the patient-specific assay on the sensitivity of ctDNA detection, we performed a simulation analysis by using smaller subsets of mutations from the patient-specific assays run in this study, and recalculated the sensitivity of ctDNA detection for pre-surgery samples (n=122) and relapse patients (n=16). Smaller number of mutations (<16) were chosen following the same prioritization and variant selection strategy as employed for the original 16-plex assays. Simulation results for ctDNA detection when using 2,4,8, or 16 of tumor mutations in the customized assays is presented in eTable 2; as expected, the sensitivity of the assay improves with an increase in the number of personalized mutations included in the assay.

eResults 2. Molecular Profiling of Metastases in Low Shedders
For two recurrence patients (ID 20 and 24), longitudinal analysis detected no ctDNA post-op (eFigure 7). We analyzed the same amount of plasma for these two patients, as for the other patients. A possible sample swap can be rejected after SNP tracer analysis, which confirmed that for each patient, the tumor, buffy coat and plasma samples came from the same individual. We performed WES of the metastatic recurrence lesions for the two patients, and confirmed that the mutations selected for plasma profiling were present in the metastases (eTable 8). WES was also performed on the metastases from patient 77. For this patient, the ctDNA longitudinal analysis did not detect ctDNA until after recurrence had been detected by radiological imaging (eFigure 7). Again, WES confirmed that the mutations selected for plasma profiling were present in the metastasis. Accordingly, we conclude that the reason for our negative post-op findings was ctDNA levels below detection level and not that the selected markers were non-informative.

eTable 1. Clinicopathological Patient Characteristics

Patient ID	Age	Gender	Primary tumor site	Primary tumor diameter, mm	UICC stage	TNMV	ACT	First relapse after OP month	Relapse site	Relapse treatment	MSS/MSI status	Perineural invasion	Number of lymph nodes in resected specimen	Microscopic radical resection	Ileus	Anastomotic leakage	Tumor perforation	WHO performance status
1	68	Male	Colon	52	II	T3N0MxV0	No	NA[a]			MSS	No	41	Yes	No	No	No	1
2	70	Male	Colon	54	II	T3N0MxV0	No	NA			MSS	Yes	31	Yes	No	No	No	1
3	67	Female	Colon	32	II	T3N0MxV0	No	NA			MSS	No	24	Yes	No	No	No	1
4	64	Male	Colon	35	III	T3N1MxV0	Yes	NA			MSS	No	39	Yes	N/A[d]	No	No	1
5	77	Male	Colon	70	II	T3N0MxV0	No	NA			MSS	No	34	Yes	No	No	No	1
6	75	Male	Colon	65	II	T3N0MxV0	No	NA			MSS	No	23	Yes	No	No	No	1
7	70	Male	Colon	29	II	T3N0M0V0	No	NA			MSS	No	28	Yes	No	No	No	1
8	65	Female	Colon	42	III	T3N1M0V0	Yes	NA			MSS	No	32	Yes	N/A	No	No	1
9	50	Male	Colon	100	II	T3N0M0V0	Yes	NA			MSS	No	51	Yes	No	N/A[d]	No	1
10	50	Female	Colon	15	III	T3N1M0V0	Yes	NA			MSS	No	27	Yes	N/A	No	No	1
11	70	Male	Colon	40	III	T3N2M0V1	Yes	NA			MSS	Yes	27	Yes	N/A	No	No	1
12	67	Female	Colon	50	II	T3N0M0V0	No	NA			MSS	No	41	Yes	No	No	No	1
13	66	Female	Colon	40	II	T4N0MxV0	Yes	NA			MSS	Yes	27	Yes	No	No	No	1
14	68	Female	Colon	63	II	T3N0MxV0	No	NA			MSI	Yes	28	Yes	No	No	No	1
15	48	Male	Colon	42	III	T3N2MxV0	Yes	NA			MSS	Yes	38	Yes	N/A	No	No	1
16	68	Female	Colon	50	II	T3N0MxV0	No	NA			MSS	Yes	28	Yes	No	No	No	1
18	67	Male	Colon	45	III	T2N2MxV0	Yes	12	Liver	Surgery	MSS	Yes	21	No	N/A	No	No	1
19	69	Female	Colon	58	II	T3N0M0V0	No	NA			MSI	Yes	28	Yes	No	No	No	2
20	73	Female	Rectal	55	III	T3N1M0V2	Yes	12	Lung	Surgery	MSS	Yes	21	Yes	N/A	No	No	1
21	68	Male	Colon	55	III	T3N1M0V0	Yes	NA			MSS	Yes	26	Yes	N/A	No	No	1
22	67	Female	Rectal	60	III	T3N1M0V1	Yes	NA			MSS	Yes	36	Yes	N/A	No	No	1
23	69	Male	Rectal	53	III	T4N1M0V1	Yes	NA			MSS	Yes	41	Yes	N/A	No	No	1
24	82	Male	Colon	77	III	T3N1M0V1	Yes	13	Liver	RFA liver	MSS	Yes	33	Yes	N/A	No	No	1
25	72	Female	Colon	95	II	T3N0M0V0	No	NA			MSI	Yes	62	Yes	No	No	No	1
26	66	Male	Colon	82	III	T4N1M0V0	Yes	NA			MSS	Yes	42	Yes	N/A	No	No	1
27	68	Male	Rectal	37	III	T3N1M0V0	Yes	NA			MSS	Yes	27	Yes	N/A	No	No	1
28	46	Female	Colon	66	III	T4N2M0V1	Yes	31	Multiple	Chemotherapy	MSS	Yes	32	Yes	N/A	No	No	1
29	65	Female	Rectal	37	III	T3N1M0V1	Yes	12	Lung	Chemotherapy	MSS	Yes	15	No	N/A	No	No	1
30	52	Female	Colon	47	III	T3N2M0V2	Yes	9	Bone	Chemotherapy	MSS	Yes	28	No	N/A	No	No	1

eTable 1. Clinicopathological Patient Characteristics

Patient ID	Age	Gender	Primary tumor site	Primary tumor diameter, mm	UICC stage	TNMV	ACT	First relapse after OP month	Relapse site	Relapse treatment	MSS/MSI status	Perineural invasion	Number of lymph nodes in resected specimen	Microscopic radical resection	Ileus	Anastomotic leakage	Tumor perforation	WHO performance status
31	54	Male	Colon	25	III	T3N1M0V0	Yes	NA			MSS	Yes	28	Yes	N/A	No	No	1
33	47	Male	Colon	170	III	T3N1M0V1	Yes	NA			MSI	NA	81	Yes	N/A	No	No	1
34	69	Male	Colon	37	II	T3N0M0V1	Yes	31	Liver	Palliative	MSS	Yes	35	Yes	No	No	No	1
35	68	Male	Colon	38	III	T3N1M0V0	Yes	NA			MSS	Yes	21	Yes	N/A	No	No	1
36	70	Male	Colon	47	III	T4N2M0V1	Yes	NA			MSS	Yes	37	Yes	N/A	No	No	1
37	75	Male	Colon	46	II	T3N0M0V0	No	6	Liver	RFA liver	MSS	Yes	30	Yes	No	No	No	1
38	49	Female	Colon	41	II	T3N0M0V0	No	NA			MSI	Yes	27	Yes	No	No	No	1
39	74	Male	Colon	115	III	T4N2M0V1	Yes	NA			MSS	Yes	27	No	N/A	No	No	1
40	69	Male	Colon	85	III	T3N2M0V1	Yes	NA			MSS	Yes	23	No	N/A	No	No	1
41	70	Male	Colon	67	II	T3N0M0V0	No	NA			MSS	Yes	35	Yes	No	No	No	1
42	60	Male	Colon	73	III	T4N2MxV1	Yes	14	Carcinosis	Chemotherapy	MSS	Yes	36	No	N/A	No	No	1
43	48	Male	Rectal	51	II	T3N0MxV0	No	NA			MSS	No	40	Yes	Yes	No	No	1
44	76	Female	Colon	70	II	T4N0M0V0	Yes	NA			MSS	No	19	Yes	No	No	No	1
45	83	Female	Colon	40	II	T3N0MxV0	No	NA			MSI	No	27	Yes	No	No	No	2
46	72	Female	Colon	70	III	T4N2MxV1	Yes	NA			MSI	No	37	No	N/A	No	Yes	2
47	73	Female	Colon	7	II	T3N0MxV0	No	NA			MSS	No	37	Yes	No	No	No	2
48	91	Female	Colon	55	III	T3N1M0V1	No	NA			MSI		32	Yes	N/A	No	No	2
49	80	Female	Colon	20	III	T4N1M0V0	Yes	NA			MSS	Yes	19	No	N/A	No	No	1
50	64	Male	Colon	83	II	T4N0M0V2	Yes	NA			MSS	Yes	40	Yes	No	No	No	1
51	60	Male	Colon	70	II	T3N0M0V0	No	NA			MSS	Yes	40	Yes	No	Yes	No	1
52	81	Male	Colon	30	II	T3N0M0V0	No	NA			MSS	No	17	Yes	No	No	No	2
53	80	Female	Colon	32	III	T3N1M0V0	No	NA			MSS	Yes	18	No	N/A	No	No	2
54	66	Male	Colon	40	III	T3N1M0V0	No	NA			MSS	Yes	17	Yes	N/A	Yes	No	NA
55	78	Male	Colon	40	II	T3N0M0V0	No	NA			MSS	Yes	18	Yes	No	No	No	2
57	50	Male	Colon	45	II	T3N0M0V0	No	NA			MSS	Yes	32	Yes	No	No	No	1
58	77	Male	Colon	52	II	T4N0M0V0	No	NA			MSI	Yes	24	Yes	No	No	No	1
59	51	Female	Colon	20	I	T2N0M0V0	No	NA			MSS	No	16	Yes	N/A	No	No	1
60	70	Female	Colon	32	II	T3N0M0V0	No	NA			MSS	Yes	14	Yes	No	No	No	1
61	67	Male	Colon	24	II	T3N0M0V0	No	NA			MSS	Yes	20	Yes	No	No	No	1
62	55	Male	Colon	30	III	T4N1M0V1	Yes	NA			MSS	No	19	Yes	N/A	No	No	1

eTable 1. Clinicopathological Patient Characteristics

Patient ID	Age	Gender	Primary tumor site	Primary tumor diameter, mm	UICC stage	TNMV	ACT	First relapse after OP month	Relapse site	Relapse treatment	MSS/MSI status	Perineural invasion	Number of lymph nodes in resected specimen	Microscopic radical resection	Ileus	Anastomotic leakage	Tumor perforation	WHO performance status
63	82	Male	Colon	40	III	T3N1MxV0	No	NA			MSI	No	27	Yes	N/A	No	No	1
64	71	Female	Colon	75	III	T3N1MxV1	Yes	NA			MSS	No	19	Yes	N/A	No	No	1
65	72	Male	Colon	55	III	T4N1MxV1	Yes	NA			MSS	Yes	31	No	N/A	No	No	1
66	72	Male	Colon	90	III	T3N2MxV0	Yes	NA			MSS	No	49	Yes	N/A	No	No	1
67	71	Male	Colon	35	III	T4N1MxV0	Yes	NA			MSS	No	31	Yes	N/A	No	No	2
68	83	Female	Colon	75	III	T3N2MxV1	No	13	Carcinosis	Chemotherapy	MSS	No	24	Yes	N/A	No	No	1
69	56	Female	Colon	25	III	T3N1MxV1	Yes	NA			MSS	No	18	Yes	N/A	No	No	2
70	66	Male	Colon	20	III	T3N2MxV0	Yes	NA			MSS	No	37	Yes	N/A	No	No	1
71	77	Male	Colon	90	III	T3N1MxV0	Yes	NA			MSI	No	26	Yes	N/A	No	No	2
72	49	Female	Colon	41	III	T3N1MxV0	Yes	NA			MSS	No	16	Yes	N/A	No	No	1
73	43	Female	Colon	64	III	T4N2MxV1	Yes	NA			MSS	No	30	Yes	N/A	No	No	1
74	72	Male	Colon	18	III	T3N2MxV0	Yes	NA			MSS	No	25	Yes	N/A	No	No	2
75	48	Female	Colon	74	III	T3N1MxV0	Yes	11	Liver	Surgery	MSS	No	48	Yes	N/A	No	No	2
76	75	Male	Colon	73	III	T3N2MxV0	Yes	NA			MSS	No	26	Yes	N/A	No	No	2
77	71	Female	Colon	52	III	T3N1MxV1	Yes	13	Lung	Surgery	MSS	Yes	280	Yes	N/A	No	No	2
78	64	Female	Colon	50	III	T4N2MxV0	Yes	NA			MSS	No	23	Yes	N/A	No	No	1
79	50	Female	Colon	40	III	T3N1MxV0	Yes	15	Liver	Surgery/RFA	MSS	No	20	Yes	N/A	No	No	1
80	73	Female	Colon	82	III	T4N1MxV0	Yes	NA			MSS	No	47	Yes	N/A	No	No	1
81	58	Female	Colon	23	III	T3N2MxV0	Yes	NA			MSS	No	17	Yes	N/A	No	No	1
82	50	Female	Colon	30	III	T3N2M0V1	Yes	11	LN[b] (throat)	Surgery	MSS	Yes	29	Yes	N/A	No	No	NA
83	62	Female	Colon	70	III	T3N2MxV0	Yes	NA			MSS	Yes	23	Yes	N/A	No	No	2
84	65	Female	Colon	100	III	T3N1MxV2	Yes	NA			MSS	No	24	Yes	N/A	No	No	1
85	61	Female	Colon	35	III	T3N2MxV0	Yes	11	Liver & lung	None	MSS	No	27	Yes	N/A	No	No	1
86	79	Male	Colon	90	III	T3N2MxV1	Yes	NA			MSS	No	30	Yes	N/A	No	No	1
87	70	Male	Colon	145	III	T3N1MxV2	Yes	NA			MSS	No	17	Yes	N/A	No	No	3
88	64	Male	Colon	60	III	T3N2MxV1	Yes	NA			MSS	No	47	Yes	N/A	No	No	1
89	75	Male	Colon	40	III	T3N2MxV0	No	17	Lung	Surgery	MSS	No	12	Yes	N/A	No	No	3
90	87	Female	Colon	62	III	T3N1MxV0	No	NA			MSS		21	No	Yes	No	No	3
91	69	Male	Colon	70	III	T3N1MxV0	Yes	NA			MSS	No	15	Yes	N/A	No	No	2
92	73	Female	Colon	100	III	T4N2MxV0	Yes	14	Local	Surgery	MSI	No	18	No	N/A	No	No	2

eTable 1. Clinicopathological Patient Characteristics

Patient ID	Age	Gender	Primary tumor site	Primary tumor diameter, mm	UICC stage	TNMV	ACT	First relapse after OP month	Relapse site	Relapse treatment	MSS/MSI status	Perineural invasion	Number of lymph nodes in resected specimen	Microscopic radical resection	Ileus	Anastomotic leakage	Tumor perforation	WHO performance status
93	81	Male	Colon	80	III	T4N2MxV1	Yes	NA			MSS	No	28	Yes	N/A	No	No	2
95	70	Male	Colon	30	I	T2N0MxV0	No	NA			MSS	No	27	Yes	N/A	No	No	1
96	64	Male	Colon	40	III	T4N2M0V1	Yes	NA			MSS	Yes	33	Yes	N/A	No	No	1
97	67	Female	Colon	60	II	T4N0MxV0	Yes	NA			MSS	No	15	Yes	No	No	No	1
98	78	Male	Colon	30	II	T3N0MxV0	No	NA			MSI	No	15	Yes	Yes	No	No	2
99	79	Male	Colon	40	III	T4N2MxV0	No	15	Multiple	Palliative	MSS	No	30	Yes	N/A	No	No	1
100	59	Female	Colon	100	II	T4N0MxV1	No	NA			MSS	Yes	12	Yes	No	No	No	2
101	72	Male	Colon	40	II	T3N0MxV2	No	NA			MSS	No	34	Yes	No	No	No	2
102	69	Male	Colon	35	I	T2N0MxV0	No	NA			MSS	No	13	Yes	N/A	No	No	1
103	70	Male	Colon	100	III	T3N1MxV1	Yes	12	Multiple	None	MSS	No	38	No	N/A	No	No	3
104	50	Male	Colon	30	III	T3N1MxV2	Yes	12	Liver	RFA liver	MSS	No	24	NA	N/A	No	No	NA
105	72	Male	Colon	70	II	T3N0MxV1	No	NA			MSS	No	36	Yes	No	No	No	2
106	70	Female	Colon	25	I	T2N0MxV0	No	NA			MSI	No	21	Yes	N/A	No	No	1
107	75	Male	Colon	30	III	T3N2MxV0	Yes	NA			MSS	No	17	Yes	N/A	No	No	1
108	72	Female	Colon	61	II	T3N0MxV0	No	12	Liver	RFA liver	MSS	No	12	Yes	No	No	No	3
109	74	Male	Colon	50	II	T3N0MxV0	No	NA			MSS	Yes	24	Yes	No	No	No	2
110	67	Male	Colon	35	I	T2N0MxV0	No	NA			MSS	No	20	Yes	N/A	No	No	2
111	52	Male	Colon	45	III	T3N2M0V1	Yes	NA			MSS	Yes	31	No	N/A	No	Yes	1
112	61	Male	Colon	40	III	T3N1MxV1	Yes	NA			MSS	Yes	25	No	N/A	No	No	1
113	78	Male	Colon	70	III	T3N1MxV1	No	NA			MSS	No	22	Yes	N/A	No	No	1
114	79	Male	Colon	60	III	T3N1MxV0	Yes	NA			MSS		33	Yes	N/A	No	No	1
115	78	Male	Colon	35	III	T3N2M0Vx	Yes	NA			MSS	No	24	No	N/A	No	No	2
116	75	Female	Colon	70	III	T4N1MxV0	Yes	NA			MSS	No	31	Yes	N/A	No	No	1
117	84	Female	Colon	56	III	T3N1MxV0	Yes	NA			MSS	No	19	No	N/A	No	No	2
118	69	Male	Colon	80	III	T4N2MxV0	Yes	NA			MSI	No	25	Yes	N/A	No	No	1
119	69	Female	Colon	60	III	T4N1MxV1	Yes	12	Local	HIPEC	MSS	Yes	29	No	N/A	No	Yes	1
120	61	Female	Colon	50	III	T3N2MxV1	Yes	NA			MSI	No	31	Yes	N/A	No	No	1
121	81	Female	Colon	20	III	T3N1MxV0	No	NA			MSS	No	14	Yes	N/A	No	No	2
122	76	Male	Colon	50	III	T3N1MxV1	Yes	NA			MSS	Yes	34	Yes	N/A	No	No	1
123	59	Male	Colon	50	III	T4N1MxV0	Yes	NA			MSS	Yes	24	Yes	N/A	No	No	1

eTable 1. Clinicopathological Patient Characteristics

Patient ID	Age	Gender	Primary tumor site	Primary tumor diameter, mm	UICC stage	TNMV	ACT	First relapse after OP month	Relapse site	Relapse treatment	MSS/MSI status	Perineural invasion	Number of lymph nodes in resected specimen	Microscopic radical resection	Ileus	Anastomotic leakage	Tumor perforation	WHO performance status
124	71	Female	Colon	47	III	T3N2MxV2	Yes	12	Multiple	Palliative	MSS	No	41	NA	N/A	No	No	2
125	78	Female	Colon	40	II	T4N0MxV0	No	12	Lung	Palliative	MSS	No	31	Yes	No	No	No	1
126	71	Male	Colon	60	II	T3N0MxV0	No	NA			MSS	No	36	Yes	No	No	No	1
127	67	Male	Colon	30	II	T3N0MxV0	No	NA			MSI	No	23	Yes	No	No	No	1
128	51	Male	Colon	8	III	T4N1M0V0	Yes	NA			MSI	Yes	54	NA	N/A	No	Yes	2
130	66	Male	Colon	60	III	T4N2M0V0	Yes	NA			MSS	Yes	19	Yes	N/A	No	No	1

[a]Not applicable
[b]Lymph node
[c]Hyperthermic intraperitoneal chemotherapy
[d]Not available

eTable 2. Performance Estimates of the Assay Based on Number of Mutations for Assay Design				
	Tracking 2 mutations	**Tracking 4 mutations**	**Tracking 8 mutations**	**Tracking 16 mutations (as reported in this study)**
Relapse prediction (out of n=16 patients)	7 (44%)	13 (81%)	13 (81%)	14 (88%)
Pre-operation ctDNA positive samples (out of n=122)	58 (48%)	82 (67%)	96 (79%)	108 (89%)

eTable 3. Patient Characteristics and Demographics of Eligible Patients		
Patients, n		125
Cancers, n		129[a]
Age (years), median (range)		69.9 (43.3-91)
Gender, n (%)	Female	52 (41.6)
	Male	73 (58.4)
Imaging follow-up (months), median (range)		12.5, (1.4-38.5)
Location, n (%)	Colon	119 (95.2)
	Rectum	6 (4.8)
Pathological UICC stage, n (%)	I	5 (4)
	II	39 (31.2)
	III	81 (64.8)
Histological type, n (%)	Adenocarcinoma	115 (92)
	Mucinous carcinoma	10 (8.7)
Histological grade, n (%)	Moderately differentiated	96 (76.8)
	Poorly differentiated	19 (15.2)
	ND	10 (8)
Adj. therapy by UICC stage, n (%)	I	0 (0.0)
	II	6 (15.3)
	III	71 (87.7)
	Total	77 (61.6)
Relapse by UICC stage, n (%)	I	0 (0.0)
	II	4 (10.2)
	III	20 (24.7)
	IV	0 (0.0)
	Total	24 (19.2)
Relapse site, n (%)[b]	Distant	23 (95.8)
	Local	1 (4.2)
MSS/MSI status, n (%)	MSS	109 (87.2)
	MSI	20 (16)
Smoking, n (%)	Never	52 (41.6)
	Former	57 (45.6)
	Current	16 (12.8)

[a]Four patients with synchronous cancers. Details available in eTable 3
[b]Details available in eTable 1

eTable 4. Summary of Samples and WES Information.

Patient ID	Source of DNA	Sample type[b]	Input, ng	WES coverage	Ti/Tv ratio	Median insert size (bp)	Percentage bases on target	Capture fold enrichment	# of variants
1	Primary tumor	FF	500	100.8	2.2	213	53.6	41.1	261
2	Primary tumor	FF	500	94.3	2.2	233	52.9	40.2	318
3	Primary tumor	FF	500	101.8	2.2	229	59	40	366
4	Primary tumor	FF	500	98.4	2.2	228	58.5	40	462
5	Primary tumor	FF	500	167.8	2.2	237	57.5	39.1	509
6	Primary tumor	FF	500	93	2.2	229	52.9	40.4	388
7	Primary tumor	FF	500	91.2	2.2	244	52.5	39.4	422
8	Primary tumor	FF	500	89.9	2.3	235	60.2	39.6	469
9	Primary tumor	FF	500	91.3	1.8	238	52.3	39.6	11342
10	Primary tumor	FF	500	140.4	2.2	204	62.8	40.5	387
11	Primary tumor	FF	500	104.3	2.3	224	66.6	40.5	448
12	Primary tumor	FF	500	106	2.3	235	58.2	39.6	594
13	Primary tumor	FF	500	47.2	2.3	244	65.6	37.9	593
14	Primary tumor	FF	500	106.6	2.2	239	59.9	39.4	14532
15	Primary tumor	FF	500	105.2	2.2	224	58.7	40.3	366
16	Primary tumor	FF	250	122.7	2.2	208	60.3	41.3	259
18	Primary tumor	FF	500	98	2.3	206	68.2	41.6	448
19	Primary tumor	FF	500	100.3	2.2	237	59.8	39.5	15574
20	Primary tumor	FF	500	112.2	2.2	233	65.4	39.8	428
20	Metastasis	FFPE	500	97.5	2.2	229	63.3	40	435
21	Primary tumor	FF	500	117	2.3	262	59.7	37.2	436
22	Primary tumor	FF	500	102.2	2.2	219	58.9	40.5	423
23	Primary tumor	FF	500	104.9	2.3	212	67.6	41.3	326
24	Primary tumor	FF	500	46.5	2.3	232	67.2	39.8	536
24	Metastasis	FFPE	500	107.4	2.3	162	64.2	45.1	645
25	Primary tumor	FF	500	106.1	2.1	247	59.1	38.8	7116
26	Primary tumor	FF	500	150.9	2.2	218	63.5	40.6	501
27	Primary tumor	FF	500	149.2	2.3	226	63.8	40.2	355
28	Primary tumor	FF	500	129.2	2.3	216	64.2	40.9	492
29	Primary tumor	FF	500	86.1	2.3	219	64.5	40.8	368
30	Primary tumor	FF	500	222	2.2	244	56.4	39.1	589
31	Primary tumor	FFPE	500	123.6	2.2	210	26.3	41.4	380
33 (S1)a	Primary tumor	FF	500	87.7	2.2	226	64.2	40.3	7628
33 (S2)a	Primary tumor	FFPE	500	65.5	2.2	164	42.5	44.4	5881

eTable 4. Summary of Samples and WES Information.

Patient ID	Source of DNA	Sample type[b]	Input, ng	WES coverage	Ti/Tv ratio	Median insert size (bp)	Percentage bases on target	Capture fold enrichment	# of variants
34	Primary tumor	FF	500	90.4	2.3	256	59.1	38.3	261
35	Primary tumor	FF	500	186.3	2.2	245	57.4	38.7	708
36	Primary tumor	FF	500	184.4	2	242	57	39	395
37	Primary tumor	FF	500	87.8	2.3	227	58.9	40	397
38	Primary tumor	FF	500	86.9	2.2	235	58.7	39.5	4657
39	Primary tumor	FF	500	179.4	2.2	242	57.7	39.1	594
40	Primary tumor	FF	500	86.4	2.3	221	63.2	40.5	558
41	Primary tumor	FF	500	88.4	2.3	216	63.6	40.8	347
42	Primary tumor	FF	500	94.9	2.2	200	62.2	41.9	361
43	Primary tumor	FF	500	121.3	2.2	227	53.1	40.5	401
44	Primary tumor	FF	500	103.8	2.3	228	61.4	40.1	434
45	Primary tumor	FF	500	107.6	2.2	228	64.4	40.2	6265
46	Primary tumor	FF	500	99	2.2	254	60.1	38.7	5943
47	Primary tumor	FF	500	94.4	2.3	209	65.3	41.5	551
48 (S1)a	Primary tumor	FF	500	95	2.1	253	60.7	38.8	7014
48 (S2)a	Primary tumor	FFPE	500	108.5	2.1	197	45.6	42.3	6276
49	Primary tumor	FF	200	127.9	2	228	60.1	40.1	308
50	Primary tumor	FF	500	93.1	2.3	225	61.2	40.3	323
51	Primary tumor	FFPE	500	130.7	2.2	219	45.3	41	525
52	Primary tumor	FFPE	500	113	2.3	187	46.2	43.5	393
53	Primary tumor	FF	500	93.7	2.3	226	64.3	40.3	286
54	Primary tumor	FF	500	113.6	2.3	228	66.3	40.1	479
55	Primary tumor	FF	500	161	2.2	258	57	38.1	342
57	Primary tumor	FF	500	85	1.8	227	64.4	40.2	20961
58	Primary tumor	FF	500	79.4	2.2	224	64.4	40.4	5804
59	Primary tumor	FF	250	127.1	2.2	214	59.6	40.9	410
60	Primary tumor	FFPE	500	119.1	2.2	199	45.6	42.4	314
61	Primary tumor	FF	500	93.6	2.2	237	60.6	39.7	423
62	Primary tumor	FFPE	500	120.2	2.3	149	38.8	46	586
63	Primary tumor	FF	100	116.6	2.2	231	61.8	38.9	7314
64	Primary tumor	FF	500	109	2.3	231	65.7	39.9	404
65	Primary tumor	FFPE	500	99.4	2.3	192	42.3	42.8	501
66	Primary tumor	FF	500	105.2	2.3	234	65.8	39.8	477
67	Primary tumor	FF	500	82.1	2.3	220	64.1	40.6	487
68	Primary tumor	FF	500	102	2.3	233	65.8	39.8	373

eTable 4. Summary of Samples and WES Information.

Patient ID	Source of DNA	Sample type[b]	Input, ng	WES coverage	Ti/Tv ratio	Median insert size (bp)	Percentage bases on target	Capture fold enrichment	# of variants
69	Primary tumor	FF	500	97.3	2.3	219	63.2	40.6	413
70	Primary tumor	FFPE	500	150.4	2.2	159	35.1	45.4	483
71	Primary tumor	FF	500	160.4	2.1	310	55.2	35.4	6173
72	Primary tumor	FF	500	126.8	2.3	229	66.1	40.1	309
73	Primary tumor	FF	500	90.3	2.3	222	64.5	40.6	432
74	Primary tumor	FF	500	127.3	2.2	207	61.7	41.3	1076
75	Primary tumor	FF	500	108.8	2.2	239	58.9	39.4	683
76	Primary tumor	FF	250	111.8	2.2	202	59	41.5	533
77	Primary tumor	FF	500	124.5	2.2	233	52.5	40.1	476
77	Metastasis	FFPE	500	118.8	2.3	196	63.8	42.7	744
78	Primary tumor	FF	500	94.2	2.3	219	67.2	41	328
79	Primary tumor	FF	500	101.2	2.3	221	66.9	40.9	263
80	Primary tumor	FF	500	93.8	2.3	243	60	39.2	646
81	Primary tumor	FF	500	101.8	2.3	227	59.4	40.2	295
82	Primary tumor	FF	500	219.9	2.2	237	59.1	39.5	415
83	Primary tumor	FFPE	200	38.6	2.4	136	39.6	46.7	609
84	Primary tumor	FFPE	500	69.2	2.3	182	41.5	43.6	371
85	Primary tumor	FF	500	107.6	2.3	219	59.2	40.6	397
86	Primary tumor	FF	200	116.2	2.2	192	60.2	41.6	630
87	Primary tumor	FF	500	120	2.2	222	52.7	40.8	477
88	Primary tumor	FF	500	192.4	2.2	233	57.5	39.7	571
89	Primary tumor	FF	500	91.7	2.3	235	61.6	39.9	611
90 (S1)a	Primary tumor	FFPE	500	94.6	2.2	187	26.8	43.4	408
90 (S2)a	Primary tumor	FFPE	500	70.8	2.1	171	68.8	44.4	480
91	Primary tumor	FF	500	82.8	2.3	225	64.1	40.4	489
92	Primary tumor	FF	500	132.1	2.1	226	61.5	40.5	6444
93	Primary tumor	FFPE	500	118.4	2.2	209	45.4	41.7	479
95	Primary tumor	FF	100	148.6	2.2	223	60.8	40.5	597
96	Primary tumor	FFPE	500	48.3	2.4	158	64.6	45.3	426
97	Primary tumor	FFPE	500	73.2	2.3	157	25.1	45.4	298
98	Primary tumor	FFPE	500	96.8	2.2	196	26.6	42.4	4659
99	Primary tumor	FFPE	500	125.1	2.2	226	44.6	40.5	503
100	Primary tumor	FF	500	81.5	2.3	199	64.7	41.8	467
101	Primary tumor	FF	500	88.1	2.3	190	64.5	42.3	359
102	Primary tumor	FFPE	500	127.7	2.3	167	44.8	45	432

eTable 4. Summary of Samples and WES Information.

Patient ID	Source of DNA	Sample type[b]	Input, ng	WES coverage	Ti/Tv ratio	Median insert size (bp)	Percentage bases on target	Capture fold enrichment	# of variants
103	Primary tumor	FF	500	169.3	2.2	227	57.8	40	596
104	Primary tumor	FF	500	99.9	2.2	242	52	39.6	247
105	Primary tumor	FF	100	383.4	2.2	222	60.6	40.1	884
106	Primary tumor	FF	500	83.2	2.2	217	64	40.8	4515
107	Primary tumor	FFPE	500	99.5	2.3	183	46.1	43.4	412
108	Primary tumor	FF	500	98.5	2.3	224	63.1	40.4	686
109	Primary tumor	FF	500	168.7	2.2	236	57.6	39.2	285
110	Primary tumor	FF	500	85.8	2.3	210	64.1	41.3	364
111	Primary tumor	FFPE	500	54.5	2.3	135	29.8	47.1	1019
112	Primary tumor	FF	500	86.5	2.3	216	67.7	40.9	315
113	Primary tumor	FFPE	500	92.2	2.3	133	32	47.6	604
114 (S1)a	Primary tumor	FF	500	80.7	2.3	243	60.3	39.2	432
114 (S2)a	Primary tumor	FF	500	98.6	2.3	248	59	38.7	603
115	Primary tumor	FF	500	179.6	2.2	244	57.8	39.1	683
116	Primary tumor	FF	500	97.6	2.3	239	57.8	39.5	436
117	Primary tumor	FF	500	91.5	2.2	228	54.8	40.7	602
118	Primary tumor	FF	500	94.4	2.2	218	66.9	41	6636
119	Primary tumor	FFPE	500	66	2.3	144	41	46.6	642
120	Primary tumor	FF	500	80.4	2.2	204	65.4	41.7	5666
121	Primary tumor	FFPE	500	94.1	2.3	137	35.1	47.7	697
122	Primary tumor	FFPE	500	61.2	2.3	143	44.6	46.4	457
123	Primary tumor	FF	500	176.9	2.2	236	58.1	39.3	525
124	Primary tumor	FF	250	137.2	2.2	220	59.5	40.5	642
125	Primary tumor	FF	500	119.6	2	214	61.7	41.1	492
126	Primary tumor	FF	500	102.6	2.2	225	54	40.7	345
127	Primary tumor	FFPE	500	83.1	2.2	136	32.9	47	7599
128	Primary tumor	FFPE	500	72.3	2.3	155	46.7	45.6	5622
130	Primary tumor	FF	500	88.8	2.3	217	65.2	40.9	452
1	Buffy coat	NA	500	41.9	2.1	247	64.7	37.5	NA[c]
2	Buffy coat	NA	500	36.5	2.3	244	64.9	37.6	NA
3	Buffy coat	NA	500	45.3	2.3	232	67.5	39.9	NA
4	Buffy coat	NA	500	45.5	2.3	240	66.7	39.2	NA
5	Buffy coat	NA	500	40.9	2.3	231	69.1	39.7	NA
6	Buffy coat	NA	500	46.1	2.3	238	67.2	39.4	NA
7	Buffy coat	NA	500	40.8	2.3	243	66.3	38.2	NA

eTable 4. Summary of Samples and WES Information.

Patient ID	Source of DNA	Sample type[b]	Input, ng	WES coverage	Ti/Tv ratio	Median insert size (bp)	Percentage bases on target	Capture fold enrichment	# of variants
8	Buffy coat	NA	500	43.2	2.3	223	68.6	40.5	NA
9	Buffy coat	NA	500	36.1	2.3	248	65.2	37.5	NA
10	Buffy coat	NA	500	41.8	2.3	230	69.6	39.9	NA
11	Buffy coat	NA	500	39.4	2.3	249	66.5	38.6	NA
12	Buffy coat	NA	500	40.8	2.3	246	65.1	37.7	NA
13	Buffy coat	NA	500	97	2.2	229	52.7	40.2	NA
14	Buffy coat	NA	500	61.3	2.3	248	64.9	37.6	NA
15	Buffy coat	NA	500	39.2	2.3	238	67.1	39.3	NA
16	Buffy coat	NA	500	92.2	2.3	267	51.5	36.3	NA
18	Buffy coat	NA	500	49.4	2.3	235	66.9	39.7	NA
19	Buffy coat	NA	500	37	2.3	243	52.8	37.6	NA
20	Buffy coat	NA	500	44.7	2.3	244	64.9	38.6	NA
20	Buffy coat	NA	500	44.7	2.3	244	64.9	38.6	NA
21	Buffy coat	NA	500	56.5	2.3	237	65.9	39.1	NA
22	Buffy coat	NA	500	54.9	2.3	234	66.2	39.4	NA
23	Buffy coat	NA	500	43.1	2.3	236	67.4	39.6	NA
24	Buffy coat	NA	500	104.2	2.3	220	66.6	40.8	NA
24	Buffy coat	NA	500	104.2	2.3	220	66.6	40.8	NA
25	Buffy coat	NA	500	43.6	2.3	253	52.7	37	NA
26	Buffy coat	NA	500	46.5	2.3	239	66.6	39.4	NA
27	Buffy coat	NA	500	54.9	2.3	241	65.7	38.9	NA
28	Buffy coat	NA	500	60.9	2.3	233	66	39.5	NA
29	Buffy coat	NA	500	45.1	2.2	234	67.3	39.7	NA
30	Buffy coat	NA	500	40	2.3	235	69.1	39.5	NA
31	Buffy coat	NA	500	56.8	2.1	233	66.1	39.4	NA
33	Buffy coat	NA	500	55.9	2.3	238	65.7	39.1	NA
34	Buffy coat	NA	500	46.8	2.3	247	52.9	37.3	NA
35	Buffy coat	NA	500	41.6	2.3	235	68.8	39.4	NA
36	Buffy coat	NA	500	36.8	2.3	235	69	39.4	NA
37	Buffy coat	NA	500	50.8	2.3	256	51.9	36.7	NA
38	Buffy coat	NA	500	54.2	2.3	252	52.6	37	NA
39	Buffy coat	NA	500	49.8	2.3	238	68.5	39.3	NA
40	Buffy coat	NA	500	62.7	2.3	280	62.1	36.4	NA
41	Buffy coat	NA	500	60.5	2.3	246	64.5	38.4	NA
42	Buffy coat	NA	500	35.9	2.3	245	65.1	37.6	NA

eTable 4. Summary of Samples and WES Information.

Patient ID	Source of DNA	Sample type[b]	Input, ng	WES coverage	Ti/Tv ratio	Median insert size (bp)	Percentage bases on target	Capture fold enrichment	# of variants
43	Buffy coat	NA	500	35.2	2.3	242	65	37.7	NA
44	Buffy coat	NA	500	45.2	2.3	238	67.2	39.5	NA
45	Buffy coat	NA	500	44.8	2.3	239	67.1	39.4	NA
46	Buffy coat	NA	500	40.6	2.3	232	67.8	39.8	NA
47	Buffy coat	NA	500	37.6	2.3	241	65.3	37.8	NA
48	Buffy coat	NA	500	52.7	2.3	228	67.9	40.1	NA
49	Buffy coat	NA	500	57.7	2.3	233	68.9	39.7	NA
50	Buffy coat	NA	500	43.2	2.3	240	52.9	37.7	NA
51	Buffy coat	NA	500	36.4	2.3	243	52.5	37.4	NA
52	Buffy coat	NA	500	55.4	2.3	244	64.5	38.5	NA
53	Buffy coat	NA	500	56	2.3	234	65.8	39.3	NA
54	Buffy coat	NA	500	79.7	2.3	239	65	38.8	NA
55	Buffy coat	NA	500	69.1	2.3	234	68.7	39.6	NA
57	Buffy coat	NA	500	72.8	2.3	250	64.1	38.2	NA
58	Buffy coat	NA	500	53.7	2.3	237	64.9	38.9	NA
59	Buffy coat	NA	500	58.4	2.3	240	64.7	38.9	NA
60	Buffy coat	NA	500	57.4	2.3	233	65.4	39.3	NA
61	Buffy coat	NA	500	81.9	2.2	236	64.8	39	NA
62	Buffy coat	NA	500	40	2.3	235	68.7	39.4	NA
63	Buffy coat	NA	500	44.8	2.1	240	67.1	39.2	NA
64	Buffy coat	NA	500	41.3	2.3	243	66.8	39.1	NA
65	Buffy coat	NA	500	39.1	2.3	233	67.5	39.7	NA
66	Buffy coat	NA	500	47.7	2.3	231	67.4	39.9	NA
67	Buffy coat	NA	500	50.2	2.3	233	67.2	39.8	NA
68	Buffy coat	NA	500	42	2.3	232	68.1	39.9	NA
69	Buffy coat	NA	500	44.9	2.3	239	67.2	39.4	NA
70	Buffy coat	NA	500	39.8	2.4	234	68.6	39.5	NA
71	Buffy coat	NA	500	39.2	2.3	232	69	39.7	NA
72	Buffy coat	NA	500	57	2.3	232	66.1	39.5	NA
73	Buffy coat	NA	500	55.3	2.1	237	66.1	39.3	NA
74	Buffy coat	NA	500	44.7	2.3	234	67.3	39.7	NA
75	Buffy coat	NA	500	36.5	2.3	239	59.5	38.3	NA
76	Buffy coat	NA	500	55.6	2.3	242	65.4	38.9	NA
77	Buffy coat	NA	500	42.1	2.3	232	67.3	40	NA
77	Buffy coat	NA	500	42.1	2.3	232	67.3	40	NA

eTable 4. Summary of Samples and WES Information.

Patient ID	Source of DNA	Sample type[b]	Input, ng	WES coverage	Ti/Tv ratio	Median insert size (bp)	Percentage bases on target	Capture fold enrichment	# of variants
78	Buffy coat	NA	500	53.5	2.3	236	66.2	39.1	NA
79	Buffy coat	NA	500	54.1	2.3	243	65.6	38.6	NA
80	Buffy coat	NA	500	44.8	2.3	237	59.9	38.6	NA
81	Buffy coat	NA	500	38.3	2.3	248	65.5	37.8	NA
82	Buffy coat	NA	500	69.3	2.3	234	68.8	39.6	NA
83	Buffy coat	NA	500	52.2	2.3	231	66.5	39.3	NA
84	Buffy coat	NA	500	39.6	2.3	244	58.8	37.9	NA
85	Buffy coat	NA	500	63.3	2.3	247	65.6	37.9	NA
86	Buffy coat	NA	500	38.3	2.3	240	59	38.1	NA
87	Buffy coat	NA	500	39.7	2.3	241	59.7	38.3	NA
88	Buffy coat	NA	500	45.4	2.3	235	68.8	39.4	NA
89	Buffy coat	NA	500	56.5	2.3	234	66.5	39.1	NA
90	Buffy coat	NA	500	41.3	2.3	234	59.9	38.8	NA
91	Buffy coat	NA	500	84	2.2	240	65.7	38.7	NA
92	Buffy coat	NA	500	54.1	2.3	235	66.1	39.1	NA
93	Buffy coat	NA	500	41.1	2.3	233	60.1	38.8	NA
95	Buffy coat	NA	500	52.3	2.1	234	66.2	39.1	NA
96	Buffy coat	NA	500	52.9	2.3	235	65.9	39	NA
97	Buffy coat	NA	500	33.1	2.3	230	60.4	38.8	NA
98	Buffy coat	NA	500	41.6	2.3	243	59.4	38.2	NA
99	Buffy coat	NA	500	38.4	2.3	245	65.7	37.9	NA
100	Buffy coat	NA	500	38.4	2.3	246	65.7	38	NA
101	Buffy coat	NA	500	43.3	2.3	247	65.1	37.6	NA
102	Buffy coat	NA	500	32.9	2.2	238	65.4	37.9	NA
103	Buffy coat	NA	500	53.5	2.3	233	68.8	39.6	NA
104	Buffy coat	NA	500	107.4	2.2	236	64	39.1	NA
105	Buffy coat	NA	500	80.5	2.2	250	52.2	37	NA
106	Buffy coat	NA	500	46.9	2.3	258	52.2	36.9	NA
107	Buffy coat	NA	500	45.6	2.3	243	65.7	38	NA
108	Buffy coat	NA	500	57.3	2.3	241	65.8	38.2	NA
109	Buffy coat	NA	500	56.5	2.3	234	68.7	39.5	NA
110	Buffy coat	NA	500	35.6	2.3	241	65	37.8	NA
111	Buffy coat	NA	500	38.8	2.3	235	60.4	38.8	NA
112	Buffy coat	NA	500	54.3	2.3	235	66.2	39.3	NA
113	Buffy coat	NA	500	78.7	2.3	237	64.9	38.9	NA

eTable 4. Summary of Samples and WES Information.

Patient ID	Source of DNA	Sample type[b]	Input, ng	WES coverage	Ti/Tv ratio	Median insert size (bp)	Percentage bases on target	Capture fold enrichment	# of variants
114	Buffy coat	NA	500	100.3	2.2	234	64.3	39.2	NA
115	Buffy coat	NA	500	38.2	2.3	236	68.7	39.4	NA
116	Buffy coat	NA	500	43.2	2.3	252	65.5	37.6	NA
117	Buffy coat	NA	500	74.2	2.3	242	65.6	38.6	NA
118	Buffy coat	NA	500	53	2.3	237	65.8	38.9	NA
119	Buffy coat	NA	500	54.9	2.3	239	65.8	38.8	NA
120	Buffy coat	NA	500	83	2.3	247	65.9	38.2	NA
121	Buffy coat	NA	500	127	2.2	225	65.9	40	NA
122	Buffy coat	NA	500	37.3	2.3	240	66.3	38.3	NA
123	Buffy coat	NA	500	56.5	2.3	234	68.7	39.5	NA
124	Buffy coat	NA	500	93.2	2.3	238	64.8	39.1	NA
125	Buffy coat	NA	500	35	2.3	236	59.9	38.5	NA
126	Buffy coat	NA	500	48.1	2.3	239	65.5	38.7	NA
127	Buffy coat	NA	500	48.5	2.3	239	66.2	38.5	NA
128	Buffy coat	NA	500	105.8	2.3	234	64.5	39.2	NA
130	Buffy coat	NA	500	50.4	2.3	248	52.9	37.2	NA

[a]Synchronous CRC were marked with S1 or S2
[b]FF=fresh frozen, FFPE=formalin fixed paraffin embedded,
[c]NA=Not applicable

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
1	0	1.2	0	24.83	0	NA[b]	8	0
1	0	3.3	0	8.65	0	NA	8.6	0
1	0	5.8	0	35.18	0	NA	8.8	0
1	0	11.5	0	47.61	0	NA	9	0
1	0	15.9	0	7.57	0	NA	7	0
1	0	18.9	0	31.62	0	NA	9	0
1	0	21.7	0	57.27	0	NA	9.1	0
1	0	24.6	0	16.54	0	NA	8.7	0
1	0	28.0	0	33.61	0	NA	9.2	0
1	0	30.8	0	45.2	0	NA	8.1	0
1	0	35.0	0	21.18	0	NA	7.5	0
2	0	-0.1	12	5.56	0.00206	(0.001-0.0031)	7.6	0.52
2	0	1.2	0	28.88	0	NA	7.8	0
2	0	2.8	0	17.26	0	NA	7.7	0
2	0	5.8	0	21.39	0	NA	8.5	0
2	0	8.8	0	29.39	0	NA	8.1	0
2	0	11.3	0	46.8	0	NA	8.5	0
2	0	15.4	0	29.16	0	NA	9.3	0
2	0	18.7	0	27.85	0	NA	9.1	0
2	0	21.4	0	37.65	0	NA	8.5	0
2	0	24.5	0	28.8	0	NA	9	0
2	0	27.6	0	40.32	0	NA	8.8	0
2	0	30.6	0	47.2	0	NA	8	0
2	0	33.3	0	22.53	0	NA	9	0
2	0	35.2	0	31.45	0	NA	7.9	0
3	0	0.0	14	31.16	0.00217	(0.0014-0.0029)	4	5.74
3	0	1.4	0	66.0	0	NA	7.8	0
3	0	4.1	0	66.0	0	NA	8.2	0
3	0	6.2	0	66.0	0	NA	8	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
3	0	9.5	0	66.0	0	NA	7.5	0
3	0	15.7	0	66.0	0	NA	8.6	0
3	0	18.1	0	50.34	0	NA	8.3	0
3	0	21.2	0	48.96	0	NA	8.9	0
3	0	24.1	0	66.0	0	NA	9.2	0
3	0	28.0	0	41.22	0	NA	7.7	0
3	0	30.9	0	37.6	0	NA	8	0
3	0	34.5	0	34.3	0	NA	8.5	0
3	0	37.8	0	50.41	0	NA	8.5	0
4	0	0.0	10	3.02	0.00170	(0.0011-0.0023)	2	0.93
4	0	1.2	0	45.18	0	NA	8	0
4	0	3.1	0	66.0	0	NA	8.3	0
4	0	5.8	0	66.0	0	NA	7.9	0
4	0	8.8	0	66.0	0	NA	8	0
4	0	11.9	0	62.18	0	NA	8	0
4	0	14.9	0	63.06	0	NA	8.5	0
4	0	17.7	0	60.1	0	NA	9	0
4	0	20.9	0	66.0	0	NA	8.9	0
4	0	24.5	0	56.46	0	NA	8.7	0
4	0	26.9	0	53.75	0	NA	8.7	0
4	0	30.1	0	54.0	0	NA	8.3	0
4	0	32.7	0	31.52	0	NA	8.7	0
4	0	35.6	0	37.34	0	NA	8	0
5	0	-0.1	16	66.0	0.01055	(0.0075-0.0136)	7.5	65.30
5	0	1.4	0	45.52	0	NA	7.2	0
5	0	3.3	0	27.32	0	NA	7.5	0
5	0	6.1	0	22.22	0	NA	8	0
5	0	9.2	0	33.08	0	NA	8	0
5	0	12.1	0	39.36	0	NA	8	0
5	0	14.8	0	26.77	0	NA	8	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
5	0	17.7	0	26.32	0	NA	9.3	0
5	0	20.5	0	33.84	0	NA	8.5	0
5	0	23.8	0	33.26	0	NA	8	0
6	0	-0.1	15	63.09	0.00164	(0.0013-0.002)	8.5	4.14
6	0	1.9	0	54.04	0	NA	8	0
6	0	3.3	0	46.39	0	NA	8	0
6	0	6.0	0	53.2	0	NA	8	0
6	0	9.2	0	32.63	0	NA	9	0
6	0	11.4	0	36.96	0	NA	8.6	0
6	0	14.6	0	66.0	0	NA	9.5	0
6	0	18.0	0	62.07	0	NA	9.3	0
6	0	21.4	0	41.71	0	NA	9.2	0
6	0	24.2	0	53.2	0	NA	9.2	0
6	0	27.5	0	29.13	0	NA	8	0
6	0	31.3	0	50.38	0	NA	8.5	0
6	0	34.6	0	47.53	0	NA	8.6	0
7	0	-0.1	0	63.8	0	NA	8.2	0
7	0	1.2	0	51.85	0	NA	8.5	0
7	0	3.3	0	43.2	0	NA	9	0
7	0	6.2	0	22.92	0	NA	9.5	0
7	0	9.0	0	45.66	0	NA	8.5	0
7	0	12.0	0	51.24	0	NA	9.5	0
7	0	14.9	0	41.52	0	NA	8.8	0
7	0	17.9	0	35.2	0	NA	8.6	0
7	0	21.0	0	36.0	0	NA	8.2	0
7	0	23.9	0	33.02	0	NA	8.9	0
7	0	27.2	0	59.72	0	NA	8.1	0
7	0	30.2	0	29.19	0	NA	9	0
7	0	33.1	0	33.45	0	NA	9	0
8	0	0.9	0	1.22	0	NA	8	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
8	0	3.0	0	66.0	0	NA	8.5	0
8	0	4.4	0	39.88	0	NA	9	0
8	0	7.6	0	29.39	0	NA	8.5	0
8	0	12.0	0	26.0	0	NA	9	0
8	0	14.7	0	34.54	0	NA	9	0
8	0	17.7	0	16.0	0	NA	9	0
8	0	20.8	0	19.16	0	NA	8.5	0
8	0	23.8	0	29.95	0	NA	7.5	0
8	0	27.1	0	34.8	0	NA	8	0
8	0	30.2	0	12.85	0	NA	8	0
8	0	33.4	0	43.6	0	NA	8.5	0
9	0	0.0	14	30.2	0.01623	(0.0106-0.0219)	8.5	19.83
9	0	0.9	0	66.0	0	NA	9	0
9	0	2.6	0	66.0	0	NA	9	0
9	0	5.2	0	66.0	0	NA	9	0
9	0	7.9	0	38.99	0	NA	8.5	0
9	0	11.5	0	66.0	0	NA	9.2	0
9	0	15.8	0	59.32	0	NA	10	0
9	0	19.2	0	42.8	0	NA	9	0
9	0	23.3	0	33.0	0	NA	8.5	0
9	0	26.9	0	47.5	0	NA	9	0
9	0	29.2	0	66.0	0	NA	8.5	0
9	0	32.7	0	66.0	0	NA	9	0
10	0	0.0	0	13.32	0	NA	8	0
10	0	2.8	0	66.0	0	NA	10	0
10	0	6.7	0	66.0	0	NA	9	0
10	0	10.2	0	66.0	0	NA	9.7	0
10	0	12.7	0	66.0	0	NA	8.7	0
10	0	16.2	0	66.0	0	NA	8.8	0
10	0	18.7	0	66.0	0	NA	8.9	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
10	0	21.9	0	66.0	0	NA	9.3	0
10	0	24.7	0	63.6	0	NA	9	0
10	0	28.1	0	66.0	0	NA	9.4	0
11	0	-0.1	0	14.17	0	NA	8	0
11	0	1.4	0	21.98	0	NA	8	0
11	0	6.1	0	66.0	0	NA	9.3	0
11	0	8.6	0	66.0	0	NA	9.3	0
11	0	12.0	0	66.0	0	NA	8.5	0
11	0	14.8	0	66.0	0	NA	9.2	0
11	0	18.2	0	66.0	0	NA	8.9	0
11	0	21.2	0	66.0	0	NA	8.7	0
11	0	24.2	0	66.0	0	NA	8.5	0
11	0	26.9	0	66.0	0	NA	8.5	0
11	0	30.5	0	66.0	0	NA	9.1	0
12	0	0.0	5	42.63	0.00033	(0.0002-0.0004)	8.3	0.53
12	0	1.5	0	28.45	0	NA	9	0
12	0	3.1	0	35.5	0	NA	9.3	0
12	0	6.1	0	26.39	0	NA	8.3	0
12	0	9.3	0	23.67	0	NA	8.7	0
12	0	12.1	0	21.54	0	NA	9.2	0
13	0	0.0	9	33.8	0.00036	(0.0002-0.0005)	8.8	0.47
13	0	1.9	0	29.68	0	NA	9	0
13	0	5.8	0	64.89	0	NA	8.6	0
13	0	8.8	0	56.76	0	NA	8.2	0
13	0	12.7	0	66.0	0	NA	8.2	0
13	0	14.8	0	28.94	0	NA	9.5	0
13	0	18.0	0	42.6	0	NA	9	0
13	0	21.4	0	66.0	0	NA	8.6	0
13	0	24.4	0	48.69	0	NA	8.3	0
13	0	27.3	0	52.46	0	NA	8.7	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
13	0	30.2	0	35.6	0	NA	8.7	0
14	0	0.0	14	30.07	0.00132	(0.0009-0.0017)	8	1.7
14	0	1.1	0	38.96	0	NA	8.6	0
14	0	3.6	0	31.47	0	NA	8.6	0
14	0	6.0	0	28.58	0	NA	9	0
14	0	8.9	0	25.51	0	NA	8.5	0
14	0	11.5	0	27.84	0	NA	7.3	0
14	0	15.0	0	41.57	0	NA	8.5	0
14	0	22.1	0	46.73	0	NA	8	0
14	0	25.3	0	57.21	0	NA	8.4	0
15	0	0.0	2	1.61	0.00761	(0.0015-0.0121)	8	0.47
15	0	1.5	0	3.62	0	NA	8	0
15	0	3.6	0	37.94	0	NA	8.4	0
15	0	5.8	0	25.76	0	NA	8	0
15	0	8.9	0	32.14	0	NA	8.9	0
15	0	12.0	0	21.03	0	NA	9	0
15	0	15.2	0	41.67	0	NA	8.7	0
15	0	18.7	0	43.74	0	NA	9	0
15	0	25.4	0	37.41	0	NA	8.6	0
15	0	29.7	0	35.74	0	NA	8.5	0
16	0	0.0	10	32.47	0.00042	(0.0003-0.0005)	9	0.46
16	0	2.1	0	31.13	0	NA	9	0
16	0	5.7	0	31.97	0	NA	9.4	0
16	0	8.5	0	36.34	0	NA	8.6	0
16	0	11.7	0	47.03	0	NA	8.6	0
16	0	15.4	0	37.2	0	NA	9	0
16	0	18.9	0	37.07	0	NA	7.5	0
16	0	21.8	0	28.0	0	NA	8.5	0
16	0	24.8	0	36.24	0	NA	8.2	0
16	0	27.8	0	43.67	0	NA	7.5	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
16	0	30.8	0	42.21	0	NA	8.5	0
18	1	0.0	16	44.0	0.00259	(0.0013-0.0039)	8	4.9
18	1	1.4	0	36.15	0	NA	8	0
18	1	3.4	0	66.0	0	NA	9	0
18	1	7.3	8	66.0	0.00034	(0.0002-0.0005)	8	1.4
18	1	10.6	5	66.0	0.00037	(0.0001-0.0006)	9	1.4
18	1	13.7	0	66.0	0	NA	8.5	0
18	1	16.4	0	66.0	0	NA	9	0
18	1	19.6	0	65.73	0	NA	9	0
18	1	21.9	0	59.13	0	NA	8.5	0
18	1	27.4	0	66.0	0	NA	8.5	0
18	1	31.1	0	66.0	0	NA	8.5	0
19	0	0.0	14	66.0	0.00197	(0.0014-0.0026)	8.8	42
19	0	1.0	0	66.0	0	NA	8	0
19	0	2.7	0	66.0	0	NA	8.5	0
19	0	6.6	0	59.7	0	NA	9.5	0
19	0	8.8	0	66.0	0	NA	9	0
19	0	11.9	0	31.27	0	NA	7.5	0
19	0	15.4	0	66.0	0	NA	9.4	0
19	0	18.2	0	66.0	0	NA	8.1	0
19	0	21.4	0	66.0	0	NA	8.2	0
19	0	24.1	0	51.56	0	NA	8.3	0
19	0	26.8	0	58.0	0	NA	8.6	0
20	1	0.0	5	26.8	0.00026	(0.0002-0.0003)	8	0.29
20	1	0.8	0	21.31	0	NA	8	0
20	1	2.7	0	30.45	0	NA	8.4	0
20	1	6.4	0	36.27	0	NA	9.5	0
20	1	8.7	0	26.81	0	NA	8.7	0
20	1	11.7	0	28.52	0	NA	9	0
20	1	15.1	0	39.12	0	NA	8.3	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
20	1	18.5	0	32.92	0	NA	8.5	0
20	1	21.1	0	66.0	0	NA	9	0
20	1	26.7	0	45.2	0	NA	9	0
21	0	0.0	13	17.12	0.00133	(0.0008-0.0019)	8	0.97
21	0	2.7	0	36.3	0	NA	8.8	0
21	0	6.1	0	60.19	0	NA	9.3	0
21	0	11.5	0	25.8	0	NA	8.2	0
21	0	15.6	0	37.12	0	NA	9.5	0
21	0	19.7	0	44.55	0	NA	9	0
21	0	24.0	0	33.05	0	NA	8.8	0
21	0	26.8	0	35.25	0	NA	8.8	0
22	0	0.0	2	1.51	0.00138	(0.0013-0.0015)	8	0.09
22	0	1.3	0	18.81	0	NA	8	0
22	0	2.2	0	14.65	0	NA	8	0
22	0	4.8	0	45.27	0	NA	7.8	0
22	0	8.2	0	59.92	0	NA	8.7	0
22	0	11.9	0	31.34	0	NA	9.4	0
22	0	14.4	0	29.39	0	NA	9.5	0
22	0	17.9	0	27.59	0	NA	8.9	0
22	0	20.6	0	31.2	0	NA	8.2	0
22	0	23.7	0	14.82	0	NA	8.4	0
22	0	26.7	0	22.68	0	NA	8.5	0
23	0	0.0	6	8.84	0.00141	(0.0005-0.0023)	8	0.53
23	0	1.1	0	7.93	0	NA	8	0
23	0	2.6	0	66.0	0	NA	9.2	0
23	0	5.8	0	53.91	0	NA	8.7	0
23	0	8.4	0	29.68	0	NA	8.9	0
23	0	11.4	0	28.8	0	NA	9.2	0
23	0	14.4	0	22.1	0	NA	9.1	0
23	0	17.2	0	43.42	0	NA	8.5	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
23	0	20.1	0	22.22	0	NA	8.5	0
23	0	23.4	0	27.81	0	NA	8.4	0
23	0	26.6	0	39.14	0	NA	8	0
24	1	0.0	11	12.4	0.00088	(0.0006-0.0012)	8	0.47
24	1	1.0	0	5.96	0	NA	8	0
24	1	3.1	0	31.4	0	NA	9	0
24	1	5.7	0	26.97	0	NA	9.3	0
24	1	8.9	0	22.3	0	NA	9	0
24	1	12.3	0	22.44	0	NA	8.6	0
24	1	14.8	0	26.4	0	NA	9.2	0
24	1	17.8	0	15.81	0	NA	8.5	0
24	1	21.7	0	19.12	0	NA	8.3	0
24	1	25.8	0	25.91	0	NA	9	0
25	0	0.0	16	45.73	0.00657	(0.0044-0.0088)	8	13
25	0	1.2	0	57.58	0	NA	8.5	0
25	0	3.1	0	30.05	0	NA	9.5	0
25	0	6.1	0	30.67	0	NA	9.5	0
25	0	8.8	0	35.19	0	NA	9.6	0
25	0	10.5	0	38.06	0	NA	9	0
25	0	14.8	0	50.93	0	NA	9	0
25	0	17.8	0	29.92	0	NA	9	0
25	0	21.2	0	50.66	0	NA	8.8	0
25	0	24.8	0	42.39	0	NA	8.4	0
25	0	28.1	0	37.63	0	NA	8.7	0
26	0	0.0	16	66.0	0.00943	(0.0072-0.0117)	8	33
26	0	1.2	0	25.77	0	NA	8	0
26	0	3.6	0	48.66	0	NA	8.9	0
26	0	6.0	0	45.85	0	NA	9	0
26	0	9.0	0	66.0	0	NA	8.7	0
26	0	12.4	0	60.83	0	NA	8.4	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
26	0	14.8	0	66.0	0	NA	9.5	0
26	0	18.2	0	66.0	0	NA	9	0
26	0	21.4	0	66.0	0	NA	8	0
26	0	24.1	0	66.0	0	NA	9	0
27	0	-0.1	14	18.4	0.00369	(0.002-0.0049)	8	2.7
27	0	1.0	0	21.71	0	NA	8	0
27	0	3.1	0	36.36	0	NA	9.2	0
27	0	6.0	0	26.0	0	NA	8.9	0
27	0	9.9	0	23.27	0	NA	9.5	0
27	0	12.0	0	23.02	0	NA	9.4	0
27	0	15.0	0	28.11	0	NA	8	0
27	0	18.0	0	29.88	0	NA	8.7	0
27	0	22.1	0	20.92	0	NA	8	0
27	0	25.3	0	30.0	0	NA	8.6	0
28	1	0.0	7	7.25	0.00069	(0.0005-0.001)	8	0.22
28	1	0.9	0	10.73	0	NA	8	0
28	1	2.8	0	66.0	0	NA	9.6	0
28	1	6.2	0	66.0	0	NA	8.9	0
28	1	9.7	0	66.0	0	NA	8.5	0
28	1	12.3	0	49.6	0	NA	8.3	0
28	1	15.2	13	22.1	0.00066	(0.0004-0.001)	8.5	0.62
28	1	18.0	16	44.93	0.00182	(0.0013-0.0024)	9.1	3.1
28	1	21.8	16	52.92	0.00777	(0.006-0.0095)	8.5	16
28	1	25.3	16	66.0	0.01076	(0.0086-0.0129)	8.3	43
29	1	0.0	0	6.74	0	NA	8	0
29	1	1.9	0	12.91	0	NA	8	0
29	1	3.4	0	39.36	0	NA	8.5	0
29	1	6.3	0	26.46	0	NA	9.3	0
29	1	10.9	11	33.2	0.00067	(0.0004-0.0009)	8.8	0.85
29	1	15.5	6	22.29	0.00041	(0.0002-0.0006)	8.4	0.38

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
29	1	17.9	13	25.51	0.00095	(0.0007-0.0012)	8.3	1.0
29	1	22.1	4	33.52	0.00042	(0.0002-0.0006)	3.3	1.4
30	1	0.0	15	14.11	0.04070	(0.0315-0.05)	8	24
30	1	1.2	15	14.15	0.07946	(0.0645-0.0947)	8	47
30	1	3.0	5	66.0	0.00013	(0.0001-0.0002)	8.5	3.2
30	1	6.0	0	66.0	0	NA	9	0
30	1	9.4	14	66.0	0.00343	(0.0026-0.0043)	9	15
30	1	13.5	15	66.0	0.33035	(0.2524-0.4076)	9.3	2743
30	1	15.6	15	66.0	0.00118	(0.0009-0.0015)	9	4.5
30	1	18.6	15	66.0	0.04747	(0.0343-0.0608)	8.7	181
31	0	0.0	4	24.63	0.00035	(0.0003-0.0004)	8	0.36
31	0	1.1	0	19.17	0	NA	8	0
31	0	3.2	0	66.0	0	NA	9.2	0
31	0	6.0	0	66.0	0	NA	8.3	0
31	0	9.3	0	66.0	0	NA	9.1	0
31	0	12.1	0	66.0	0	NA	8.8	0
31	0	15.1	0	66.0	0	NA	9.1	0
31	0	18.2	0	66.0	0	NA	8.3	0
31	0	21.3	0	49.92	0	NA	8.2	0
31	0	25.0	0	43.5	0	NA	8.2	0
33	0	0.0	9	12.73	0.00992	(0.0025-0.0174)	6	7.2
33	0	1.1	7	16.8	0.00718	(0.0024-0.01)	8	4.4
33	0	2.8	0	58.2	0	NA	8	0
33	0	5.6	0	54.16	0	NA	9	0
33	0	8.7	0	39.02	0	NA	9	0
33	0	9.9	0	51.56	0	NA	8	0
33	0	11.7	0	47.92	0	NA	9.5	0
33	0	15.3	0	40.8	0	NA	8.4	0
33	0	18.3	0	32.01	0	NA	8.5	0
33	0	21.9	0	29.17	0	NA	8.7	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
34	1	0.0	12	34.4	0.00049	(0.0004-0.0006)	9.3	0.62
34	1	1.2	0	31.75	0	NA	9.2	0
34	1	2.6	0	41.44	0	NA	8.5	0
34	1	5.3	0	34.79	0	NA	8.5	0
34	1	8.5	0	35.36	0	NA	9	0
34	1	11.2	0	66.0	0	NA	8.9	0
34	1	14.0	7	29.6	0.00051	(0.0004-0.0007)	8.4	0.61
34	1	17.1	14	31.76	0.00185	(0.0015-0.0022)	8.5	2.4
34	1	20.5	14	42.63	0.08891	(0.0768-0.1009)	8.5	152
35	0	0.0	12	66.0	0.00042	(0.0003-0.0005)	9.4	1.5
35	0	0.9	0	57.65	0	NA	8.1	0
35	0	2.0	0	37.32	0	NA	8.7	0
35	0	6.1	0	66.0	0	NA	9	0
35	0	9.0	0	45.58	0	NA	9	0
35	0	11.9	0	40.29	0	NA	8.5	0
35	0	15.1	0	55.17	0	NA	8.8	0
35	0	18.3	0	56.49	0	NA	9	0
36	0	0.0	15	62.06	0.00098	(0.0006-0.0013)	8.9	2.3
36	0	1.0	0	66.0	0	NA	9.5	0
36	0	3.0	0	66.0	0	NA	9.5	0
36	0	6.3	0	66.0	0	NA	9.2	0
36	0	8.9	0	65.38	0	NA	9	0
36	0	11.8	0	51.1	0	NA	8.7	0
36	0	14.7	0	41.29	0	NA	8.9	0
36	0	18.0	0	66.0	0	NA	9	0
37	1	0.0	16	49.2	0.00582	(0.0043-0.0073)	8.8	11
37	1	1.7	16	36.8	0.00440	(0.0035-0.0053)	9.5	5.8
37	1	3.0	16	14.44	0.01216	(0.0091-0.0154)	9.2	6.53
37	1	5.8	16	55.16	0.00609	(0.0044-0.0078)	8.4	14
37	1	9.0	0	32.66	0	NA	9	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
37	1	12.2	0	29.99	0	NA	8.6	0
37	1	15.2	0	29.62	0	NA	8.5	0
37	1	18.5	0	31.6	0	NA	8.6	0
38	0	0.0	7	4.29	0.00147	(0.0007-0.0022)	8.8	0.25
38	0	1.0	0	30.25	0	NA	9	0
38	0	4.5	0	27.15	0	NA	8.5	0
38	0	6.5	0	36.41	0	NA	9	0
38	0	9.4	0	29.05	0	NA	8.9	0
38	0	12.1	0	20.8	0	NA	8.5	0
38	0	16.8	0	16.79	0	NA	8.3	0
39	0	0.0	16	40.4	0.00508	(0.0041-0.0061)	9.6	7.3
39	0	1.0	0	66.0	0	NA	8.9	0
39	0	3.9	0	49.73	0	NA	9.5	0
39	0	6.5	0	66.0	0	NA	9.1	0
39	0	9.7	0	49.02	0	NA	8.5	0
39	0	12.3	0	50.25	0	NA	9	0
39	0	15.9	0	39.6	0	NA	8.5	0
39	0	18.7	0	36.58	0	NA	7.9	0
40	0	0.0	10	41.6	0.00027	(0.0002-0.0004)	9.2	0.40
40	0	0.9	0	48.23	0	NA	9	0
40	0	3.3	0	66.0	0	NA	9.2	0
40	0	6.2	0	66.0	0	NA	9.2	0
40	0	9.4	0	66.0	0	NA	9	0
40	0	11.8	0	66.0	0	NA	9.3	0
41	0	0.0	16	35.1	0.00819	(0.0061-0.0103)	9	11
41	0	0.5	0	54.27	0	NA	9	0
41	0	3.0	0	54.62	0	NA	9	0
41	0	6.2	0	28.8	0	NA	8.5	0
41	0	11.8	0	28.23	0	NA	8.8	0
42	1	0.0	10	27.29	0.00060	(0.0005-0.0007)	7.3	0.76

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
42	1	2.5	0	66.0	0	NA	7.8	0
43	0	-0.1	16	27.63	0.00226	(0.0016-0.003)	6.8	3.1
43	0	2.6	0	33.39	0	NA	7.5	0
44	0	0.0	2	39.8	0.00026	NA	4	0.78
44	0	1.8	0	37.67	0	NA	8	0
45	0	0.0	0	1.0	0	NA	8	0
45	0	2.3	0	44.8	0	NA	9	0
46	0	-0.1	4	8.4	0.00182	(0.0008-0.0025)	4	1.2
46	0	1.7	0	36.56	0	NA	9.4	0
47	0	0.0	16	66.0	0.00815	(0.0061-0.0102)	9.1	23
47	0	3.4	0	45.97	0	NA	7.3	0
48	0	0.0	0	6.8	0	NA	8	0
48	0	0.4	0	4.64	0	NA	8	0
49	0	0.0	2	42.4	0.00019	(0.0002-0.0002)	8	0.33
49	0	0.4	0	66.0	0	NA	7.5	0
50	0	0.0	12	41.96	0.00150	(0.0008-0.0022)	8.8	2.4
50	0	0.5	0	66.0	0	NA	9.1	0
51	0	0.0	16	28.4	0.01376	(0.0101-0.0175)	8	17
51	0	0.5	0	66.0	0	NA	8.2	0
52	0	-0.3	15	66.0	0.00112	(0.0008-0.0014)	8.5	4.7
52	0	0.6	0	45.2	0	NA	8	0
53	0	0.0	15	62.4	0.00561	(0.0044-0.0069)	9.2	13
53	0	0.2	0	66.0	0	NA	8.5	0
54	0	0.0	16	66.0	0.00155	(0.0012-0.002)	9.6	19
54	0	0.5	0	66.0	0	NA	8.8	0
55	0	0.0	5	65.26	0.00059	(0.0001-0.0011)	8.8	1.5
55	0	0.4	0	66.0	0	NA	9	0
57	0	0.0	7	46.2	0.00095	(0.0004-0.0015)	9	1.7
57	0	0.9	0	66.0	0	NA	9.5	0
58	0	0.0	8	61.6	0.00025	(0.0002-0.0003)	8.5	0.63

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
58	0	1.0	0	66.0	0	NA	8.2	0
59	0	0.0	0	10.8	0	NA	9	0
59	0	0.9	0	66.0	0	NA	9.3	0
60	0	0.0	12	50.8	0.00043	(0.0003-0.0006)	9.4	0.79
60	0	0.9	0	66.0	0	NA	9.4	0
61	0	0.0	10	33.46	0.00090	(0.0006-0.0012)	8.5	1.2
61	0	0.6	0	65.6	0	NA	8.8	0
62	0	-0.3	8	2.37	0.00168	(0.0005-0.0029)	8	0.17
62	0	0.7	5	9.77	0.00063	(0.0004-0.0008)	8	0.26
62	0	2.8	0	66.0	0	NA	8.6	0
62	0	6.0	0	43.39	0	NA	8.5	0
62	0	9.0	0	28.95	0	NA	8.7	0
62	0	12.5	0	31.6	0	NA	7	0
62	0	15.1	0	21.27	0	NA	8.6	0
62	0	17.7	0	27.62	0	NA	8.2	0
62	0	21.1	0	41.97	0	NA	8.6	0
62	0	23.7	0	50.25	0	NA	9	0
62	0	26.7	0	31.62	0	NA	8.5	0
62	0	29.7	0	37.6	0	NA	8.5	0
63	0	-0.2	12	13.09	0.00926	(0.0052-0.0134)	8	5.2
63	0	0.9	0	66.0	0	NA	8	0
63	0	3.5	0	55.57	0	NA	9	0
63	0	5.6	0	47.87	0	NA	9.5	0
63	0	9.2	0	43.09	0	NA	8.8	0
63	0	12.4	0	61.6	0	NA	8.7	0
63	0	15.0	0	31.29	0	NA	8.7	0
63	0	18.2	0	43.22	0	NA	8.2	0
63	0	21.0	0	51.11	0	NA	8.8	0
63	0	23.5	0	66.0	0	NA	8.5	0
63	0	26.6	0	45.19	0	NA	7.8	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
63	0	30.1	0	42.4	0	NA	8.2	0
64	0	-0.2	3	2.94	0.00133	(0.0009-0.0017)	8	0.16
64	0	0.7	0	8.26	0	NA	8	0
64	0	3.1	0	66.0	0	NA	8.4	0
64	0	6.0	0	66.0	0	NA	9.5	0
64	0	9.6	0	43.65	0	NA	8.5	0
64	0	11.8	0	45.8	0	NA	8.5	0
64	0	15.5	0	28.37	0	NA	8.3	0
64	0	18.0	0	52.38	0	NA	8.7	0
64	0	21.3	0	59.28	0	NA	8	0
64	0	24.0	0	56.33	0	NA	8.4	0
64	0	26.6	0	41.2	0	NA	8	0
64	0	30.0	0	30.33	0	NA	8	0
65	0	-0.3	8	10.4	0.00135	(0.0007-0.002)	8	0.60
65	0	0.7	0	12.86	0	NA	8	0
65	0	2.9	0	66.0	0	NA	9	0
65	0	6.0	0	66.0	0	NA	9	0
65	0	8.8	0	66.0	0	NA	9	0
65	0	11.6	0	41.77	0	NA	8.7	0
66	0	-0.2	3	3.49	0.00318	(0-0.008)	8	0.54
66	0	0.6	0	14.88	0	NA	8	0
66	0	3.0	0	66.0	0	NA	8.8	0
66	0	6.0	0	43.8	0	NA	8.7	0
66	0	9.0	0	42.25	0	NA	9	0
66	0	11.8	0	27.01	0	NA	9.1	0
66	0	15.2	0	32.46	0	NA	8.5	0
66	0	18.2	0	38.0	0	NA	8	0
66	0	21.0	0	24.79	0	NA	8.5	0
66	0	24.0	0	27.48	0	NA	8.8	0
66	0	27.4	0	35.25	0	NA	8	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
66	0	30.2	0	51.16	0	NA	8.4	0
67	0	-0.3	10	23.24	0.00064	(0.0004-0.0008)	8	0.63
67	0	0.9	0	23.2	0	NA	8	0
67	0	2.8	0	66.0	0	NA	8.6	0
67	0	5.7	0	66.0	0	NA	9	0
67	0	8.7	0	66.0	0	NA	8.5	0
67	0	11.5	0	55.68	0	NA	8.5	0
67	0	15.0	0	66.0	0	NA	8.3	0
67	0	18.0	0	66.0	0	NA	8	0
67	0	20.8	0	66.0	0	NA	8.5	0
67	0	24.0	0	66.0	0	NA	8.6	0
67	0	27.6	0	66.0	0	NA	8	0
67	0	30.0	0	66.0	0	NA	8.5	0
68	1	-0.2	14	15.9	0.00142	(0.0008-0.002)	8	0.96
68	1	1.0	0	13.93	0	NA	8	0
68	1	3.1	6	19.33	0.00044	(0.0003-0.0006)	8.9	0.32
68	1	6.1	2	31.81	0.00030	NA	8.5	0.34
68	1	9.4	11	31.08	0.00075	(0.0005-0.0009)	9	0.86
68	1	12.0	16	40.71	0.00218	(0.0017-0.0026)	8.8	3.4
68	1	15.0	16	35.09	0.01345	(0.0106-0.0164)	8.8	18
68	1	18.4	16	25.56	0.00420	(0.0029-0.0055)	8.5	4.3
68	1	21.6	16	8.02	0.00516	(0.0037-0.0065)	8.5	1.7
69	0	-0.2	8	12.35	0.00065	(0.0004-0.0008)	8	0.31
69	0	0.9	0	5.37	0	NA	8	0
69	0	3.0	0	66.0	0	NA	8.2	0
69	0	6.0	0	66.0	0	NA	8.7	0
69	0	8.9	0	40.17	0	NA	8.6	0
69	0	12.0	0	33.33	0	NA	8.5	0
69	0	15.2	0	34.23	0	NA	8.5	0
69	0	18.0	0	30.15	0	NA	8.3	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
69	0	21.0	0	50.25	0	NA	8.5	0
69	0	23.9	0	43.27	0	NA	8	0
69	0	27.1	0	50.93	0	NA	8	0
70	0	-0.3	3	19.69	0.00048	(0.0001-0.0009)	8	0.41
70	0	0.9	0	36.35	0	NA	8	0
70	0	3.0	0	66.0	0	NA	8.5	0
70	0	6.1	0	66.0	0	NA	8.7	0
70	0	8.9	0	42.8	0	NA	8.5	0
70	0	12.0	0	26.12	0	NA	8.4	0
70	0	15.0	0	34.62	0	NA	8.5	0
70	0	18.1	0	37.67	0	NA	8.5	0
70	0	20.9	0	42.63	0	NA	8.4	0
71	0	-0.2	2	1.65	0.01366	(0-0.0294)	8	0.78
71	0	0.7	0	12.28	0	NA	8	0
71	0	3.3	0	66.0	0	NA	8.6	0
71	0	6.2	0	66.0	0	NA	8.4	0
71	0	9.2	0	52.1	0	NA	9.5	0
71	0	12.1	0	63.2	0	NA	9	0
71	0	14.7	0	66.0	0	NA	8.8	0
71	0	18.4	0	48.83	0	NA	8	0
71	0	21.0	0	40.4	0	NA	8.5	0
71	0	23.9	0	47.73	0	NA	8	0
71	0	26.4	0	66.0	0	NA	8.3	0
72	0	-0.4	16	26.13	0.00127	(0.001-0.0015)	9	1.2
72	0	0.8	0	1.64	0	NA	8	0
72	0	3.3	0	22.71	0	NA	9	0
72	0	6.0	0	35.07	0	NA	8.9	0
72	0	9.0	0	29.06	0	NA	9	0
72	0	12.1	0	23.14	0	NA	8.3	0
72	0	15.1	0	26.86	0	NA	8.9	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
72	0	18.0	0	28.95	0	NA	8.7	0
72	0	21.0	0	40.03	0	NA	8.5	0
72	0	24.0	0	30.8	0	NA	9	0
73	0	-0.3	0	12.68	0	NA	8	0
73	0	0.8	0	15.2	0	NA	8	0
73	0	2.9	0	66.0	0	NA	9.4	0
73	0	5.9	0	66.0	0	NA	8.4	0
73	0	8.4	0	66.0	0	NA	8.8	0
73	0	12.1	0	66.0	0	NA	8.5	0
73	0	15.3	0	62.03	0	NA	8.6	0
73	0	18.1	0	66.0	0	NA	8	0
73	0	21.4	0	66.0	0	NA	8.5	0
73	0	24.1	0	61.87	0	NA	8.7	0
74	0	-0.2	2	30.66	0.00053	(0-0.0011)	8	0.69
74	0	0.5	0	20.8	0	NA	8	0
74	0	3.7	0	60.49	0	NA	8.7	0
74	0	6.2	0	52.95	0	NA	8	0
74	0	9.5	0	63.08	0	NA	8.5	0
74	0	12.2	0	66.0	0	NA	8.3	0
74	0	14.8	0	59.77	0	NA	8.2	0
74	0	18.0	0	66.0	0	NA	8	0
74	0	21.4	0	56.84	0	NA	8.5	0
75	1	-0.2	14	15.81	0.02285	(0.008-0.0379)	8	16
75	1	0.5	0	66.0	0	NA	8	0
75	1	3.2	15	26.0	0.10138	(0.0395-0.1637)	4	225
75	1	5.8	15	66.0	0.11723	(0.0519-0.1829)	8.7	311
75	1	8.6	15	66.0	0.20991	(0.122-0.2986)	8	857
75	1	11.7	15	66.0	0.42629	(0.3125-0.5401)	8	8240
75	1	14.7	0	34.88	0	NA	6.5	0
75	1	18.2	0	32.87	0	NA	8.2	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
75	1	20.3	2	27.53	0.00036	(0.0003-0.0004)	8	0.43
75	1	24.4	0	60.4	0	NA	8	0
76	0	-0.2	0	1.0	0	NA	8	0
76	0	0.9	0	36.5	0	NA	8	0
76	0	3.0	0	66.0	0	NA	8	0
76	0	5.9	0	66.0	0	NA	8	0
76	0	8.2	0	66.0	0	NA	8.8	0
76	0	12.2	0	66.0	0	NA	9	0
76	0	14.9	0	66.0	0	NA	8.4	0
76	0	17.7	0	66.0	0	NA	8.5	0
76	0	20.5	0	66.0	0	NA	8.5	0
76	0	23.3	0	66.0	0	NA	8.4	0
77	1	-0.2	2	26.27	0.00065	(0.0003-0.001)	8	0.73
77	1	0.7	0	56.18	0	NA	8	0
77	1	3.2	0	66.0	0	NA	8.6	0
77	1	5.7	0	66.0	0	NA	8.9	0
77	1	8.9	0	43.65	0	NA	8.5	0
77	1	12.2	0	61.7	0	NA	9.1	0
77	1	14.8	0	66.0	0	NA	7.5	0
77	1	17.7	2	66.0	0.00014	(0.0001-0.0002)	9	2.00
77	1	20.5	0	66.0	0	NA	8	0
78	0	-0.2	9	66.0	0.00034	(0.0002-0.0005)	8.6	1.6
78	0	0.8	0	66.0	0	NA	8.3	0
78	0	2.6	0	66.0	0	NA	8.6	0
78	0	6.0	0	47.6	0	NA	8.6	0
78	0	9.1	0	40.4	0	NA	8.5	0
78	0	12.7	0	43.6	0	NA	8.2	0
78	0	15.9	0	33.6	0	NA	8.8	0
78	0	18.5	0	40.4	0	NA	8.3	0
79	1	-0.1	15	10.4	0.00870	(0.0062-0.0112)	8.2	3.8

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
79	1	0.6	15	25.6	0.00299	(0.002-0.0039)	8.5	3.1
79	1	3.4	13	31.6	0.00124	(0.0008-0.0017)	8.2	1.6
79	1	6.4	15	40.0	0.00330	(0.0025-0.0042)	8.5	5.3
79	1	9.2	15	24.8	0.03044	(0.0217-0.0393)	8.9	29
79	1	12.2	15	30.8	0.04347	(0.0306-0.0565)	8	57
79	1	15.7	15	25.6	0.14889	(0.1118-0.1863)	7.8	167
79	1	17.8	6	30.8	0.00051	(0.0001-0.0009)	8	0.68
80	0	-0.2	16	56.49	0.00112	(0.0008-0.0015)	9	2.4
80	0	0.9	0	66.0	0	NA	9	0
80	0	3.1	0	66.0	0	NA	8.9	0
80	0	5.7	0	66.0	0	NA	8.8	0
80	0	9.0	2	66.0	0.00024	(0.0002-0.0003)	9	0.75
80	0	12.0	0	45.17	0	NA	8.9	0
80	0	15.2	0	48.37	0	NA	8.5	0
80	0	18.0	0	62.02	0	NA	8.5	0
81	0	-0.2	5	66.0	0.00023	(0.0001-0.0003)	8.7	0.82
81	0	0.6	0	66.0	0	NA	8.4	0
81	0	3.1	0	66.0	0	NA	8.1	0
81	0	5.9	0	66.0	0	NA	8.7	0
81	0	8.9	0	66.0	0	NA	7.8	0
81	0	12.0	0	66.0	0	NA	8.9	0
81	0	14.7	0	66.0	0	NA	8.4	0
81	0	17.8	0	66.0	0	NA	9.5	0
82	1	-0.3	16	13.6	0.00880	(0.0066-0.011)	8	5.1
82	1	0.4	13	25.55	0.00291	(0.0022-0.0036)	9	2.8
82	1	3.4	14	45.41	0.00104	(0.0008-0.0013)	8.9	1.8
82	1	7.3	15	26.58	0.00804	(0.0063-0.0098)	8.5	8.5
82	1	11.3	15	21.6	0.03572	(0.0277-0.0437)	8.6	31
83	0	-0.2	15	44.4	0.00050	(0.0003-0.0007)	8	0.93
83	0	0.5	0	47.6	0	NA	8.5	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
83	0	2.8	0	66.0	0	NA	9	0
83	0	6.0	0	66.0	0	NA	8.7	0
83	0	9.2	0	56.8	0	NA	8.5	0
83	0	12.6	0	66.0	0	NA	8.5	0
83	0	14.8	0	50.8	0	NA	8.2	0
84	0	-0.2	15	66.0	0.00423	(0.0031-0.0054)	8	39
84	0	0.9	0	66.0	0	NA	8.5	0
84	0	2.9	0	53.2	0	NA	8.7	0
84	0	6.4	0	40.0	0	NA	8.5	0
84	0	8.9	0	50.0	0	NA	8	0
84	0	15.0	0	37.6	0	NA	8	0
85	1	-0.2	12	27.2	0.00512	(0.0034-0.0069)	8.2	5.8
85	1	0.4	11	66.0	0.00635	(0.0048-0.0079)	8.6	18
85	1	3.2	11	60.08	0.01166	(0.009-0.0144)	7.3	33
85	1	6.6	11	66.0	0.09014	(0.073-0.1078)	8	729
85	1	8.7	11	66.0	0.39998	(0.3217-0.4803)	8	4347
85	1	12.2	11	66.0	0.53680	(0.4215-0.6526)	9.5	70790
86	0	-0.2	16	52.0	0.02194	(0.0175-0.0265)	9.1	43
86	0	0.3	0	66.0	0	NA	10	0
87	0	-0.2	16	35.85	0.00378	(0.0033-0.0043)	8.4	5.5
87	0	0.5	0	49.6	0	NA	8.5	0
88	0	-0.2	8	13.52	0.00207	(0.0012-0.0029)	9	1.1
88	0	0.5	0	31.41	0	NA	9	0
89	1	-0.2	0	66.0	0	NA	8.6	0
89	1	0.5	0	66.0	0	NA	9.3	0
90	0	-0.1	2	62.0	0.00210	(0-0.006)	8.3	0.26
90	0	0.3	0	66.0	0	NA	9.1	0
91	0	-0.4	16	66.0	0.00173	(0.0012-0.0023)	8.5	6.4
91	0	0.3	0	66.0	0	NA	8.5	0
92	1	-0.3	13	66.0	0.00896	(0.0061-0.0119)	9.4	22

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
92	1	0.4	0	66.0	0	NA	8.8	0
93	0	-0.2	14	29.6	0.00110	(0.0008-0.0014)	8.5	1.3
93	0	0.5	0	49.2	0	NA	9	0
95	0	-0.2	10	39.18	0.00064	(0.0004-0.0009)	8.9	0.86
95	0	0.3	0	66.0	0	NA	8.5	0
96	0	-0.2	14	50.4	0.00057	(0.0004-0.0007)	9	1.1
96	0	0.4	0	66.0	0	NA	9	0
97	0	-0.2	10	20.8	0.00096	(0.0006-0.0011)	8	0.78
97	0	0.4	0	66.0	0	NA	8	0
98	0	-0.2	0	51.2	0	NA	9	0
98	0	0.4	0	66.0	0	NA	8.2	0
99	1	-0.2	13	66.0	0.00065	(0.0002-0.0011)	8.4	2.2
99	1	0.3	0	66.0	0	NA	9.1	0
100	0	-0.2	7	23.6	0.00046	(0.0003-0.0007)	8.4	0.45
100	0	0.3	0	66.0	0	NA	8.7	0
101	0	-0.2	16	66.0	0.00292	(0.0021-0.0038)	8.5	9.7
101	0	0.4	0	66.0	0	NA	8.2	0
102	0	-0.3	13	44.0	0.00044	(0.0003-0.0006)	8.4	0.78
102	0	0.4	0	66.0	0	NA	8.6	0
103	1	-0.1	16	33.58	0.03621	(0.0219-0.0505)	8.5	49
103	1	0.3	0	66.0	0	NA	8.8	0
104	1	-0.1	16	16.29	0.00220	(0.0017-0.0027)	8.5	1.5
104	1	0.4	4	28.15	0.00043	(0.0003-0.0006)	8.8	0.47
105	0	-0.2	16	34.0	0.00861	(0.0059-0.0114)	8.5	12
105	0	0.4	0	66.0	0	NA	8.7	0
106	0	-0.2	0	33.2	0	NA	8.5	0
106	0	0.5	0	66.0	0	NA	9	0
107	0	-0.3	5	35.2	0.00045	(0.0002-0.0007)	8.5	0.65
107	0	0.5	0	66.0	0	NA	8.7	0
108	1	-0.4	3	66.0	0.00041	(0.0001-0.0007)	9	1.5

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
108	1	0.3	0	66.0	0	NA	8.8	0
109	0	-0.1	6	36.04	0.00068	(0.0003-0.0011)	9	0.93
109	0	0.3	0	66.0	0	NA	8.9	0
110	0	-0.2	0	62.8	0	NA	8.7	0
110	0	0.6	0	63.6	0	NA	8.8	0
111	0	0.0	16	49.47	0.00542	(0.0023-0.0086)	8	12
111	0	3.1	0	66.0	0	NA	8	0
111	0	5.8	0	66.0	0	NA	8	0
111	0	9.3	0	41.78	0	NA	9	0
111	0	11.8	0	41.17	0	NA	8.5	0
111	0	15.5	0	53.71	0	NA	8.2	0
111	0	18.3	0	51.93	0	NA	8.8	0
111	0	21.0	0	39.2	0	NA	8	0
111	0	27.5	0	21.96	0	NA	8.5	0
112	0	0.0	3	25.32	0.00045	(0.0003-0.0006)	8	0.47
112	0	0.9	0	14.55	0	NA	8	0
112	0	3.1	0	65.59	0	NA	8	0
112	0	6.3	0	66.0	0	NA	8	0
112	0	11.2	0	46.05	0	NA	8.5	0
112	0	15.7	0	66.0	0	NA	7.9	0
112	0	18.2	0	34.65	0	NA	8	0
112	0	21.6	0	25.46	0	NA	7.8	0
112	0	27.2	0	42.97	0	NA	8	0
113	0	0.0	16	40.69	0.00320	(0.0022-0.0043)	8	5.6
113	0	1.2	0	9.51	0	NA	8	0
113	0	3.9	0	37.2	0	NA	8.8	0
113	0	6.7	0	52.0	0	NA	8.5	0
113	0	9.5	0	56.4	0	NA	8.3	0
113	0	11.6	0	51.2	0	NA	8.3	0
113	0	15.0	0	60.8	0	NA	7.8	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
113	0	18.2	0	43.6	0	NA	8	0
113	0	21.3	0	52.8	0	NA	9	0
114	0	-0.2	4	13.59	0.00513	NA	8	2.6
114	0	0.7	0	19.37	0	NA	8	0
114	0	3.2	0	66.0	0	NA	8.8	0
114	0	6.0	0	54.4	0	NA	7.8	0
114	0	9.0	0	52.24	0	NA	8	0
114	0	12.7	0	65.62	0	NA	8.6	0
114	0	15.2	0	66.0	0	NA	8.5	0
114	0	18.0	0	57.84	0	NA	6.5	0
115	0	0.0	12	66.0	0.00067	(0.0003-0.001)	8.8	1.9
115	0	0.7	0	56.82	0	NA	8.5	0
115	0	2.9	0	49.39	0	NA	7.8	0
115	0	5.7	0	60.52	0	NA	8	0
115	0	8.5	0	54.15	0	NA	7.7	0
115	0	11.9	0	59.2	0	NA	8.5	0
115	0	15.1	0	66.0	0	NA	8.3	0
115	0	18.1	0	66.0	0	NA	8.5	0
116	0	-0.1	10	55.23	0.00076	(0.0006-0.0009)	9	1.6
116	0	0.5	0	66.0	0	NA	8.5	0
116	0	3.5	0	50.35	0	NA	7.5	0
116	0	6.5	0	66.0	0	NA	8	0
116	0	9.3	0	66.0	0	NA	9	0
116	0	12.0	0	66.0	0	NA	8.5	0
116	0	15.0	0	59.45	0	NA	7.5	0
116	0	18.2	0	65.2	0	NA	9	0
117	0	-0.1	6	30.74	0.00064	(0.0002-0.0011)	7.5	0.89
117	0	0.7	0	66.0	0	NA	4.3	0
118	0	0.0	14	27.2	0.00142	(0.0007-0.0021)	8.6	1.51
118	0	0.9	0	63.6	0	NA	8.5	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
118	0	3.0	0	66.0	0	NA	8.4	0
118	0	5.7	0	66.0	0	NA	8.8	0
118	0	9.0	0	66.0	0	NA	6.5	0
118	0	12.4	0	61.2	0	NA	7	0
118	0	15.1	0	66.0	0	NA	8.2	0
119	1	-0.1	15	59.6	0.00118	(0.0009-0.0015)	8.5	2.8
119	1	0.9	12	66.0	0.00054	(0.0003-0.0007)	8.5	4.3
119	1	3.5	6	50.4	0.00026	(0.0002-0.0004)	8.2	0.53
119	1	6.2	0	66.0	0	NA	8.4	0
119	1	11.1	8	66.0	0.00022	(0.0002-0.0003)	8.7	0.61
119	1	13.4	13	60.8	0.00075	(0.0005-0.001)	9	1.7
120	0	0.0	14	17.6	0.00654	(0.0044-0.0087)	8	4.9
120	0	0.5	0	14.0	0	NA	8.1	0
120	0	3.4	0	66.0	0	NA	7	0
120	0	6.4	0	66.0	0	NA	8.2	0
120	0	9.0	0	19.6	0	NA	8.5	0
121	0	0.0	0	66.0	0	NA	9.2	0
121	0	0.8	0	66.0	0	NA	7.1	0
122	0	0.0	11	66.0	0.00060	(0.0004-0.0008)	8.4	7.7
122	0	0.7	0	66.0	0	NA	8.5	0
123	0	0.0	5	42.13	0.00054	(0.0001-0.001)	8.5	0.53
123	0	0.4	0	66.0	0	NA	8.5	0
123	0	3.0	0	66.0	0	NA	8.1	0
123	0	6.2	0	66.0	0	NA	8.5	0
123	0	9.2	0	35.58	0	NA	7.8	0
123	0	11.7	0	48.02	0	NA	8.5	0
124	1	0.0	16	66.0	0.04668	(0.0396-0.0542)	9	375
124	1	0.5	13	48.87	0.00074	(0.0005-0.0009)	8	1.5
124	1	3.1	7	43.62	0.00054	(0.0003-0.0007)	8.4	0.91
124	1	5.8	13	39.78	0.00063	(0.0005-0.0008)	9	0.96

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months)[a]	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
124	1	8.8	16	51.85	0.03123	(0.026-0.0367)	8.5	65
124	1	12.0	16	44.17	0.23820	(0.2037-0.2736)	9	398
125	1	-0.2	15	32.16	0.00140	(0.0011-0.0017)	8.5	1.8
125	1	0.7	0	38.8	0	NA	7.5	0
126	0	-0.2	13	51.65	0.00772	(0.0028-0.0131)	8.2	17
126	0	0.5	0	44.72	0	NA	7.6	0
127	0	0.0	15	66.0	0.00136	(0.0008-0.002)	9.1	12
127	0	0.6	0	66.0	0	NA	8.3	0
128	0	21.3	0	62.4	0	NA	8.5	0
128	0	24.0	0	42.4	0	NA	8.5	0
128	0	27.0	0	62.4	0	NA	8.3	0
128	0	30.0	0	44.8	0	NA	8.4	0
128	0	33.2	0	57.2	0	NA	8.4	0
128	0	36.6	0	46.0	0	NA	8.1	0
130	0	0.0	14	50.48	0.00099	(0.0006-0.0014)	9.6	1.9
130	0	0.4	2	66.0	0.00019	(0-0.0005)	9.8	0.98

[a]A negative value indicates that the blood was drawn on a day prior to surgery. A null value indicates that the sampling timepoint is on the same day as surgery, but prior to surgery.
[b]Not applicable

eTable 6. Recurrence-Free Survival Analysis by Clinicopathological Variables and Post-op ctDNA Status at Day 30

Variable	Univariate analysis			Multivariate analysis			
	HR	(95% CI)	*P*-value	HR	(95% CI)	*P*-value	Schoenfeld *P*-value
All patients with a day 30 postoperative sample (n = 94)							
Age							
< mean versus ≥ Mean	1.4	(0.52-3.8)	.51				
Stage							
Stage II versus stage III	5.3	(1.2-23.0)	**.028**	2.4	(0.48-12.2)	.29	
Tumor site							
Right versus left	1.9	(0.72-5.3)	.19				
Lymphovascular invasion							
No versus yes	2.7	(1.0-7.0)	**.044**	1.9	(0.63-5.6)	.26	
MMR-status							
Deficient versus proficient	0.45	(0.059-3.4)	.43				
Radical resection (micro)							
Yes versus no	2.3	(0.72-7.2)	.16				
Histology							
Adeno- versus mucinouscarcinoma	1.6	(0.35-7.2)	.53				
Tumor differentiation							
Medium/well versus poor	0.86	(0.19-3.8)	.85				
Gender							
Female versus male	0.24	(0.079-0.74)	**.013**[a]	.21	(0.06-0.69)	**.010**	
ctDNA status up to 6 weeks after OP (no ACT)							
ctDNA- versus ctDNA+	7.2	(2.7-19.0)	**<.001**	4.5	(1.6-12.8)	**.004**	
Global goodness-of fit test for Cox proportional hazards models							.22

[a]The present consecutive cohort unexpectedly has an extreme high proportion of relapse events among women 28.8% (15/52) and an a low proportion among men 12.3% (9/73). In the Danish population the overall relapse rates in women and men, in the period from 2001 to 2011, were 22% (2233/9958) and 25% (2803/11194), respectively.[13] Hence, the association between female sex and relapse observed in the present cohort is likely false.

eTable 7. Recurrence-Free Survival Analysis by Clinicopathological Variables, Post-op ctDNA, and Post-op CEA Status at First Timepoint Post-ACT

Variable	Univariate analysis			Multivariate analysis			
	HR	(95% CI)	*P*-value	HR	(95% CI)	*P*-value	Schoenfeld *P*-value
All patients with longitudinally collected plasma and ACT (n=58)							
Age							
< mean versus ≥ Mean	1.3	(0.44-3.8)	.61				
Stage							
Stage II versus stage III	1.3	(0.17-10.0)	.79				
Tumor site							
Right versus left	1.8	(0.63-5.3)	.27				
Lymphovascular invasion							
No versus yes	3.0	(0.93-9.5)	.066				
MMR-status							
Deficient versus proficient	1.1	(0.0-Inf)	>.99				
Radical resection (micro)							
Yes versus no	2.6	(0.77-9)	.13				
Histology							
Adeno- versus mucinouscarcinoma	1.1	(.14-8.3)	.94				
Tumor differentiation							
Medium/well versus poor	1.2	(0.27-5.6)	.78				
Tumor perforation							
No versus yes	0.91	(0.12-7)	.93				
Gender							
Female versus male	0.2	(0.055-0.71)	**.013**[a]	0.37	(0.09-1.5)	.16	
CEA							
CEA- versus CEA+	2.4	(0.74-7.9)	.14				
ctDNA							
ctDNA- versus ctDNA+	17.5	(5.4-56.5)	**<.001**	11.8	(3.4-40.8)	**<.001**	
Global goodness-of fit test for Cox proportional hazards models							.80

[a]The present consecutive cohort unexpectedly has an extreme high proportion of relapse events among women 28.8% (15/52) and an a low proportion among men 12.3% (9/73). In the Danish population the overall relapse rates in women and men, in the period from 2001 to 2011, were 22% (2233/9958) and 25% (2803/11194), respectively.[13] Hence, the association between female sex and relapse observed in the present cohort is likely false.

eTable 8. Matched Tumor and Metastatic WES

	Patient ID		
	20[a]	24[a]	77[b]
Mutations present in tumor also present in the metastasis (%)	62.6	50.0	79.4
Mutations screened in plasma also present in the metastasis (%)	100.0	87.5	93.8

[a]No ctDNA detected
[b]ctDNA detected after relapse

eTable 9. Recurrence-Free Survival Analysis by Clinicopathological Variables and Post-op ctDNA and CEA Status in Surveillance Samples

Variable	Univariate analysis			Multivariate analysis			
	HR	(95% CI)	*P*-value	HR	(95% CI)	*P*-value	*Schoenfeld P-value*
All patients with longitudinally collected plasma (n=75)							
Age							
< mean versus ≥ Mean	1.0	(0.39-2.8)	.95				
Stage							
Stage II versus stage III	3.3	(0.74-15)	.12				
Tumor site							
Right versus left	1.2	(0.45-3.2)	.72				
Lymphovascular invasion							
No versus yes	4.1	(1.4-12)	**.010**	1.1	(0.35-3.4)	.87	
MMR-status							
Deficient versus proficient	1.2	(0.0-Inf)	>.99				
Radical resection (micro)							
Yes versus no	2.9	(0.88-9.3)	.080				
Histology							
Adeno- versus mucinous carcinoma	0.84	(.84-6.4)	.87				
Tumor differentiation							
Medium/well versus poor	0.92	(0.21-4.1)	.91				
Tumor perforation							
No versus yes	1.1	(0.15-8.7)	.89				
Gender							
Female versus male	0.25	(0.08-0.8)	**.018**[a]	0.94	(0.28-3.2)	.92	
CEA							
CEA- versus CEA+	2.8	(0.95-8.0)	.061				
ctDNA							
ctDNA- versus ctDNA+	43.5	(9.8-193.5)	**<.001**	39.9	(7.5-211.0)	**<.001**	
Global goodness-of fit test for Cox proportional hazards models							.84

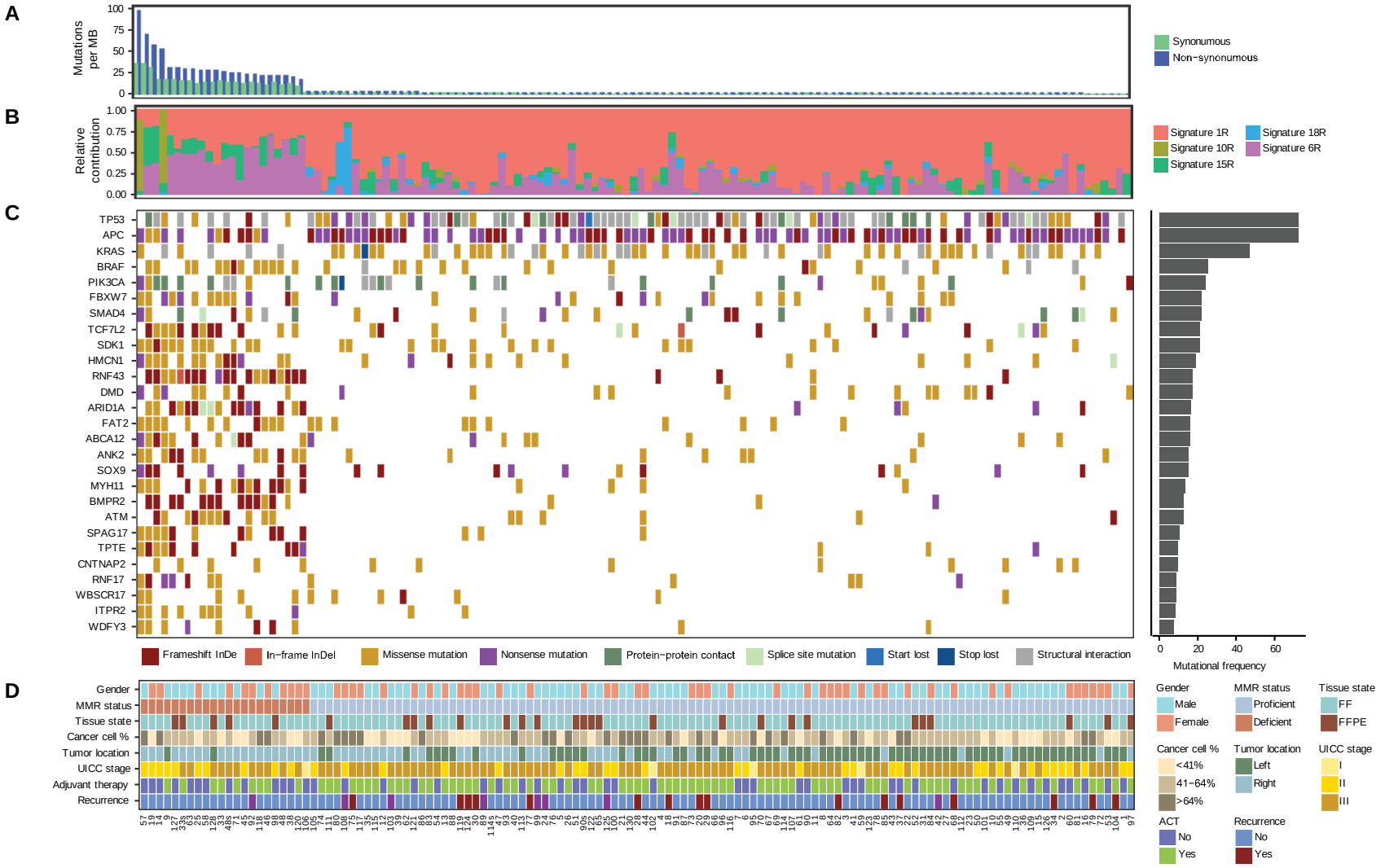
[a]The present consecutive cohort unexpectedly has an extreme high proportion of relapse events among women 28.8% (15/52) and an a low proportion among men 12.3% (9/73). In the Danish population the overall relapse rates in women and men, in the period from 2001 to 2011, were 22% (2233/9958) and 25% (2803/11194), respectively.[13] Hence, the association between female sex and relapse observed in the present cohort is likely false.

eTable 10. Patients With Actionable Mutations Detected in the Primary Tumor

Pt. ID	Chr	Gene	POS (Hg19)	SNP ID	Nucleotide	Alt. nucleotide	POS change	Aminoacid change	Cancer type	Drug	Database	ctDNA+ longitudinal plasma
42	chr14	*AKT1*	105246551	rs121434592	C	T	c.49G>A	p.Glu17Lys	Solid Tumors	AZD5363	https://www.mycancergenome.org/	
42	chr7	*BRAF*	140453136	rs113488022	A	T	c.1799T>A	p.Val600Glu	CRC	RTK-Inhibitor+Trametinib	http://oncokb.org/#/actionableGenes	
18	chr12	*KRAS*	25398284	rs121913529	C	T	c.35G>A	p.Gly12Asp	All Tumors	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	Yes
20	chr12	*KRAS*	25398284	rs121913529	C	T	c.35G>A	p.Gly12Asp	All Tumors	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	
20	chr10	*PTEN*	89692981	.	T	TG	c.988dupG	p.Glu330fs	CRC	GSK2636771 & AZD8186	http://oncokb.org/#/actionableGenes	
68	chr3	*PIK3CA*	178936091	rs104886003	G	A	c.1633G>A	p.E545K	All Tumors	AZD8186	https://www.mycancergenome.org/	Yes
68	chr12	*KRAS*	25398284	rs121913529	C	T	c.35G>A	p.Gly12Asp	All Tumors	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	Yes
24	chr12	*KRAS*	25398284	rs121913529	C	A	c.35G>T	p.Gly12Val	CRC	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	
24	chr18	*SMAD4*	48604706	.	G	T	c.1528G>T	p.Gly510*	All Tumors		https://www.mycancergenome.org/	
29	chr12	*KRAS*	25398281	rs112445441	C	T	c.38G>A	p.Gly13Asp	CRC	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	Yes
30	chr3	*PIK3CA*	178936082	.	G	A	c.1624G>A	E542K	CRC/Breast cancer	Aspirin/PI3K inhibitors	https://www.mycancergenome.org/	Yes
30	chr12	*KRAS*	25398284	rs121913529	C	A	c.35G>T	p.Gly12Val	CRC/Breast cancer	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	Yes
30	chr18	*SMAD4*	48591928	rs377767350	T	G	c.1091T>G	p.Leu364Trp	CRC		https://www.mycancergenome.org/	Yes
75	chr17	*ERBB2*	37880261	.	G	T	c.2305G>T	p.Asp769Tyr	All Tumors	Neratinib/afatinib	https://civicdb.org	Yes
75	chr17	*ERBB2*	37881000	rs121913471	G	T	c.2329G>T	p.Val777Leu	All Tumors	Neratinib/afatinib	https://civicdb.org	Yes
77	chr7	*BRAF*	140453136	rs113488022	A	T	c.1799T>A	p.Val600Glu	CRC	RTK-Inhibitor+Trametinib	http://oncokb.org/#/actionableGenes	Yes
37	chr12	*KRAS*	25398285	rs121913530	C	T	c.34G>A	p.Gly12Ser	CRC	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	Yes
89	chr12	*KRAS*	25398285	rs121913530	C	A	c.34G>T	p.Gly12Cys	CRC	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	
125	chr12	*KRAS*	25398284	rs121913529	C	A	c.35G>T	p.Gly12Val	CRC	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	
85	chr3	*PIK3CA*	178938934	.	G	A	c.2176G>A	p.Glu726Lys	CRC	Aspirin/PI3K inhibitors	https://www.mycancergenome.org/	Yes
85	chr12	*KRAS*	25398285	rs121913530	C	A	c.34G>T	p.Gly12Cys	All Tumors	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	Yes
119	chr12	*KRAS*	25398285	rs121913530	C	T	c.34G>A	p.Gly12Ser	CRC	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	Yes

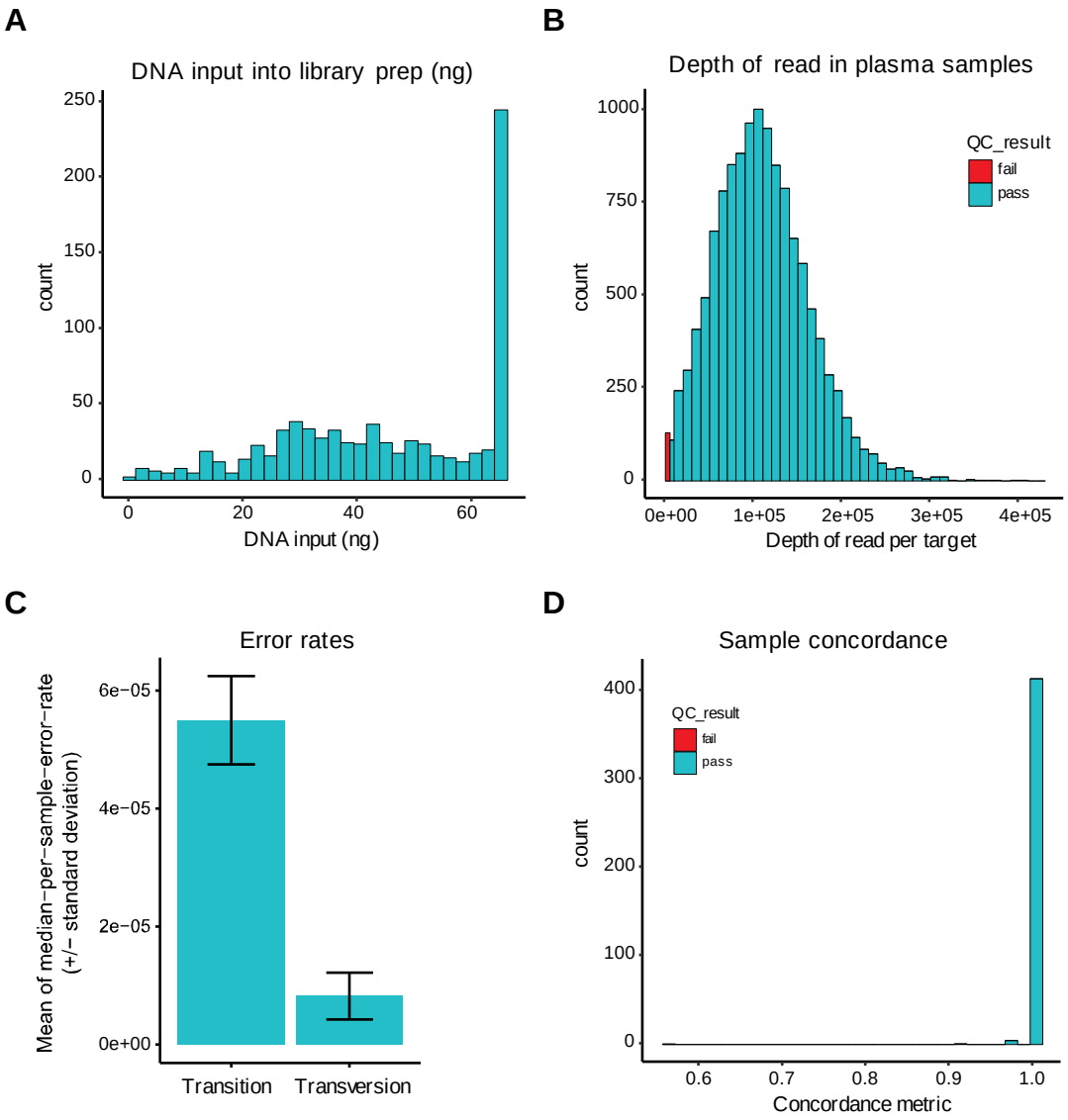
99	chr12	*KRAS*	25380278	.	A	T	c.180T>A	p.Gly60Gly	CRC	GDC-0994, KO-947, LY3214997	http://oncokb.org/#/actionableGenes	
99	chr12	*KRAS*	25380277	rs121913238	G	T	c.181C>A	p.Gln61Lys	CRC	GDC-0994, KO-947, LY3214998	http://oncokb.org/#/actionableGenes	
104	chr18	*SMAD4*	48604701	.	G	C	c.1523G>C	p.Gly508Ala	CRC		https://www.mycancergenome.org/	Yes
124	chr7	*BRAF*	140453136	rs113488022	A	T	c.1799T>A	p.Val600Glu	CRC	RTK-Inhibitor+Trametinib	http://oncokb.org/#/actionableGenes	Yes
108	chr3	*PIK3CA*	178936094	rs121913286	C	A	c.1636C>A	Q546K	CRC	Aspirin/PI3K inhibitors	https://www.mycancergenome.org/	
108	chr12	*KRAS*	25398285	rs121913530	C	A	c.34G>T	p.Gly12Cys	CRC	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	

eFigure 1. Summary of Clinical, Histopathological, and Molecular Parameters for All 125 Patients. A) Rate of synonymous and non-synonymous mutations called from WES. B) The relative contribution of the five most prevalent colorectal cancer associated mutational signatures. C) Mutations in frequently mutated genes in colorectal cancer.[11] D) Clinical and histopathological characteristics.

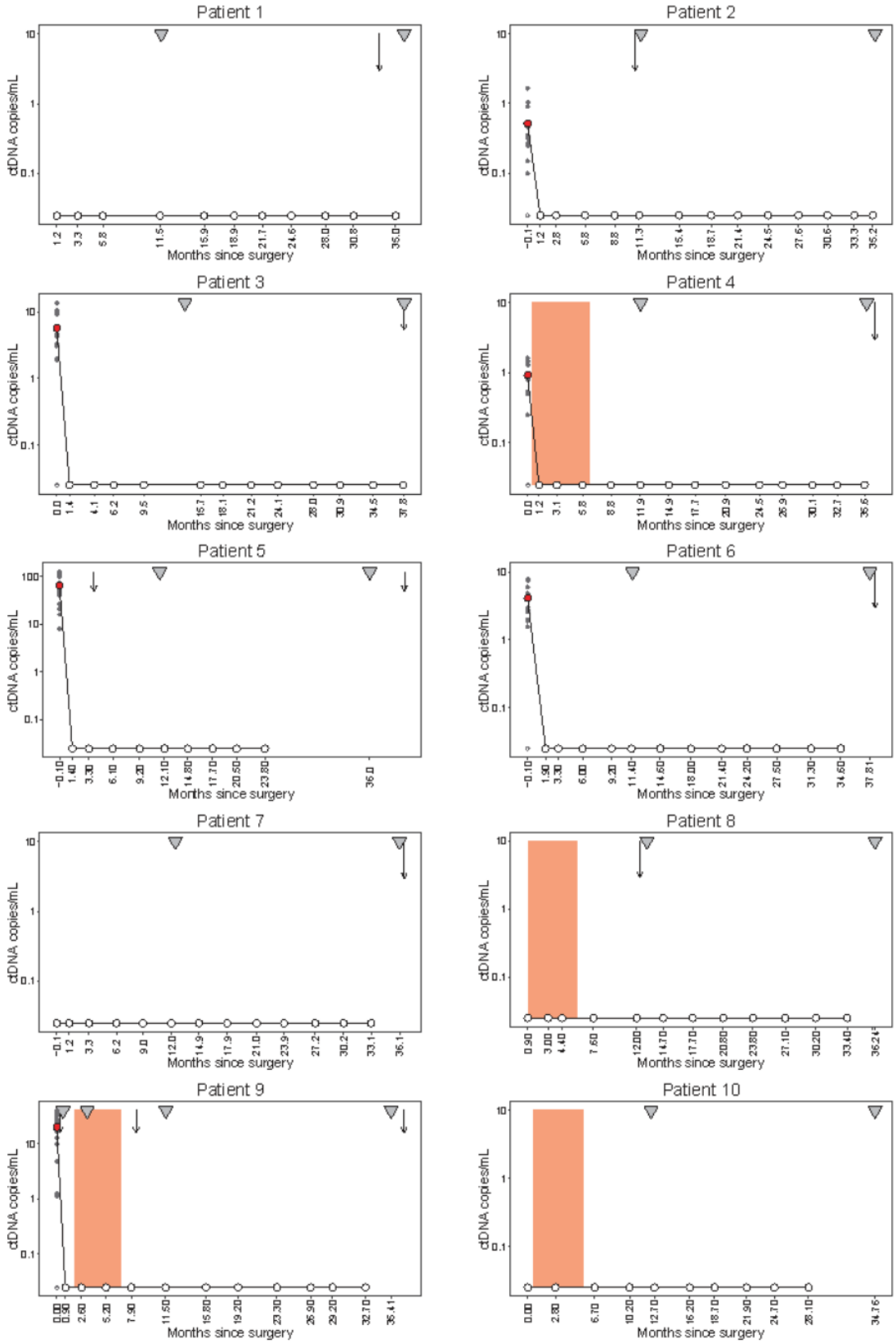


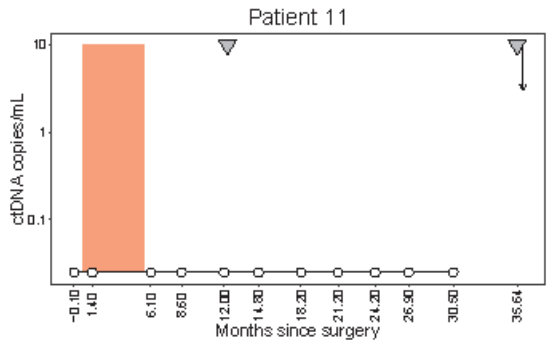
eFigure 2. Quality Control Metrics for cfDNA Sequencing Using Multiplex PCR NGS.

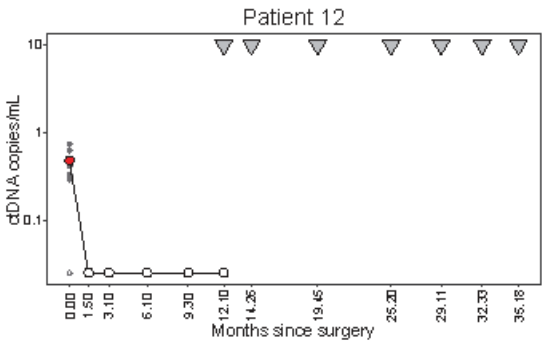
A) DNA input into NGS libraries. A maximum of 66ng was used for library preparation (approximately 20.000 genome equivalents). B) read depth of amplicons. Amplicons with coverage less than 5000x were excluded from analyses and samples with less than 8 passing amplicons (out of 16) failed sequencing coverage QC. C) mean error rates. D) Sequencing sample concordance between plasma samples from the same patient as well as the corresponding tissue biopsy sample. All plasma samples from the relapse patients were tested and no mixup was identified.

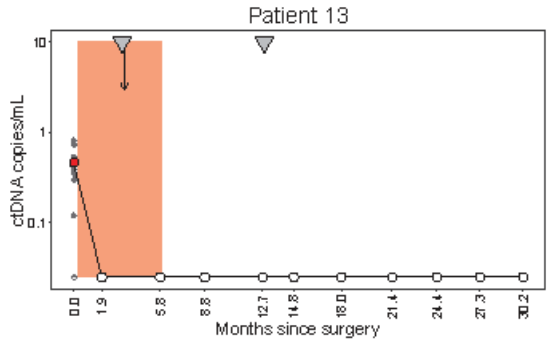


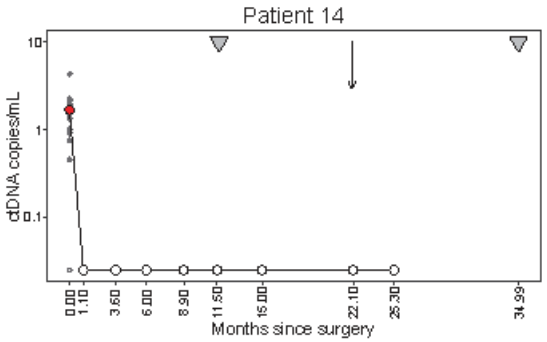
eFigure 3. Detailed ctDNA Results and Disease Course Information. Representation of detailed disease courses, applied treatments and longitudinal ctDNA analyses for all 125 patients. ctDNA is represented as copies/mL plasma for each mutation individually and as a mean of all detected mutations.

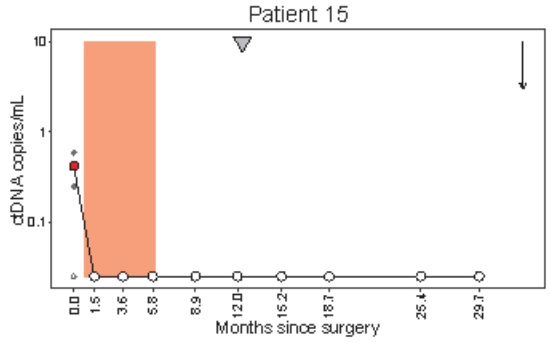


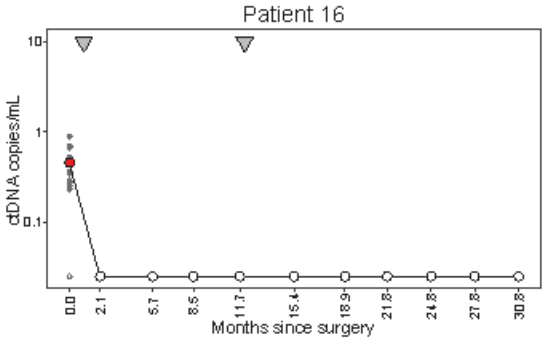


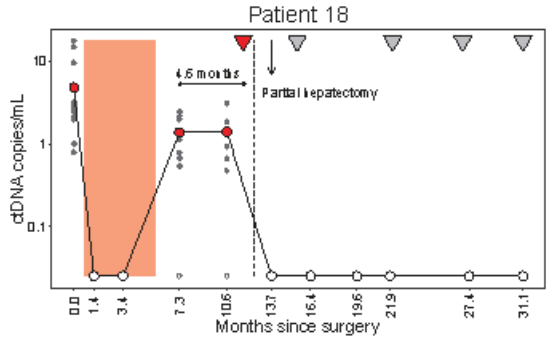


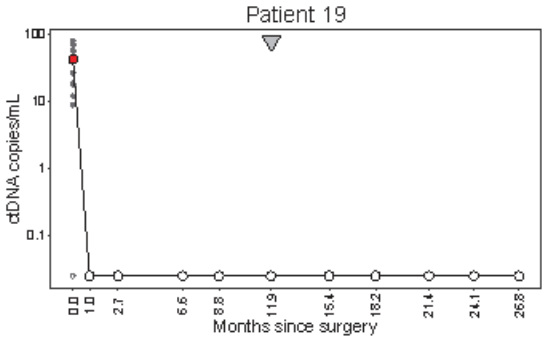


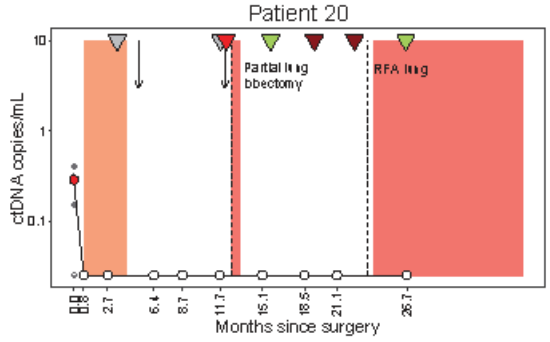


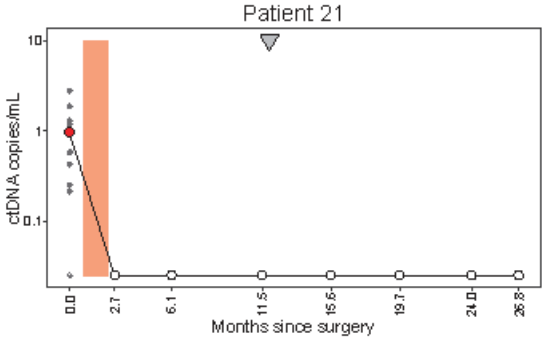


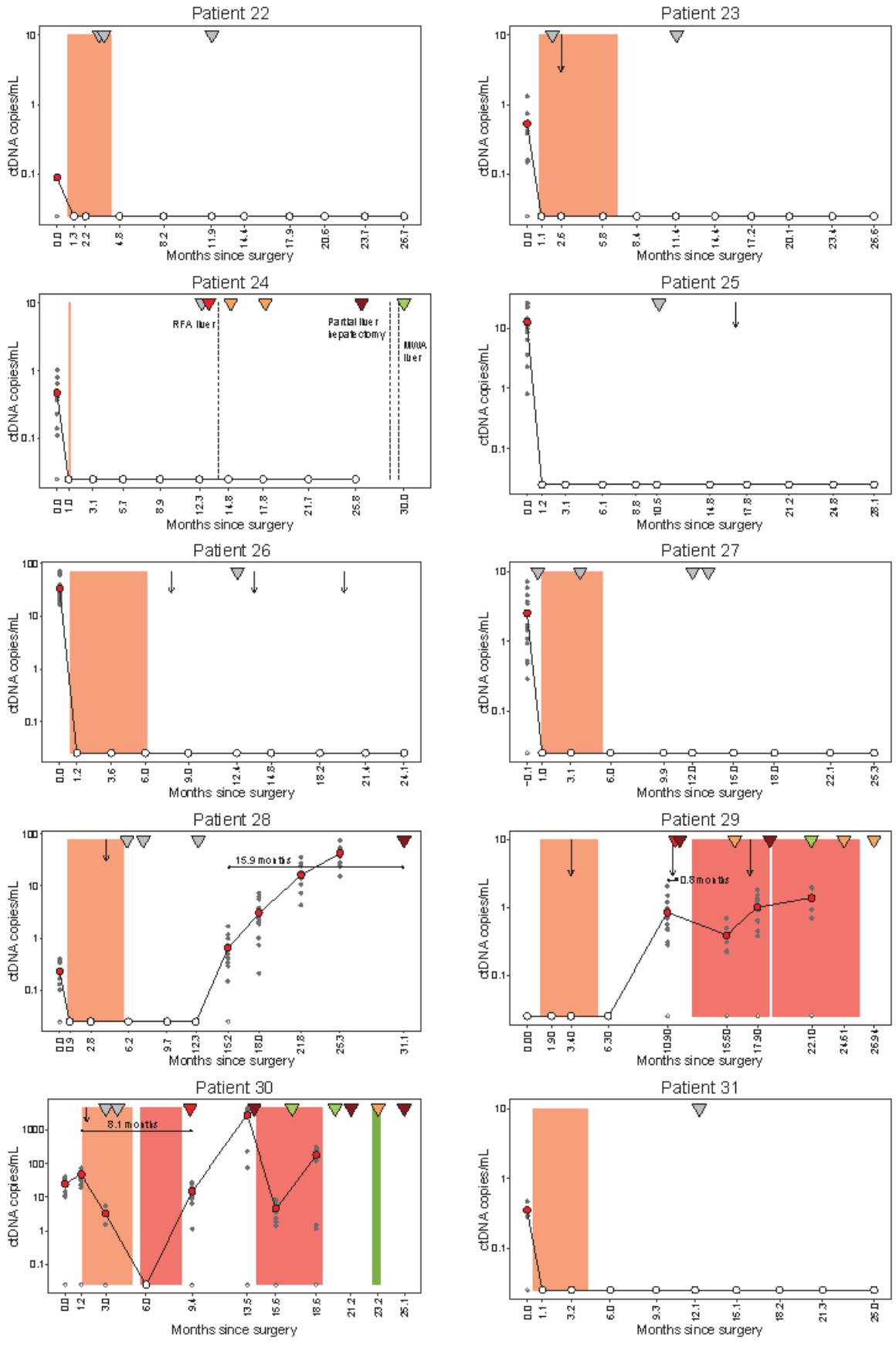


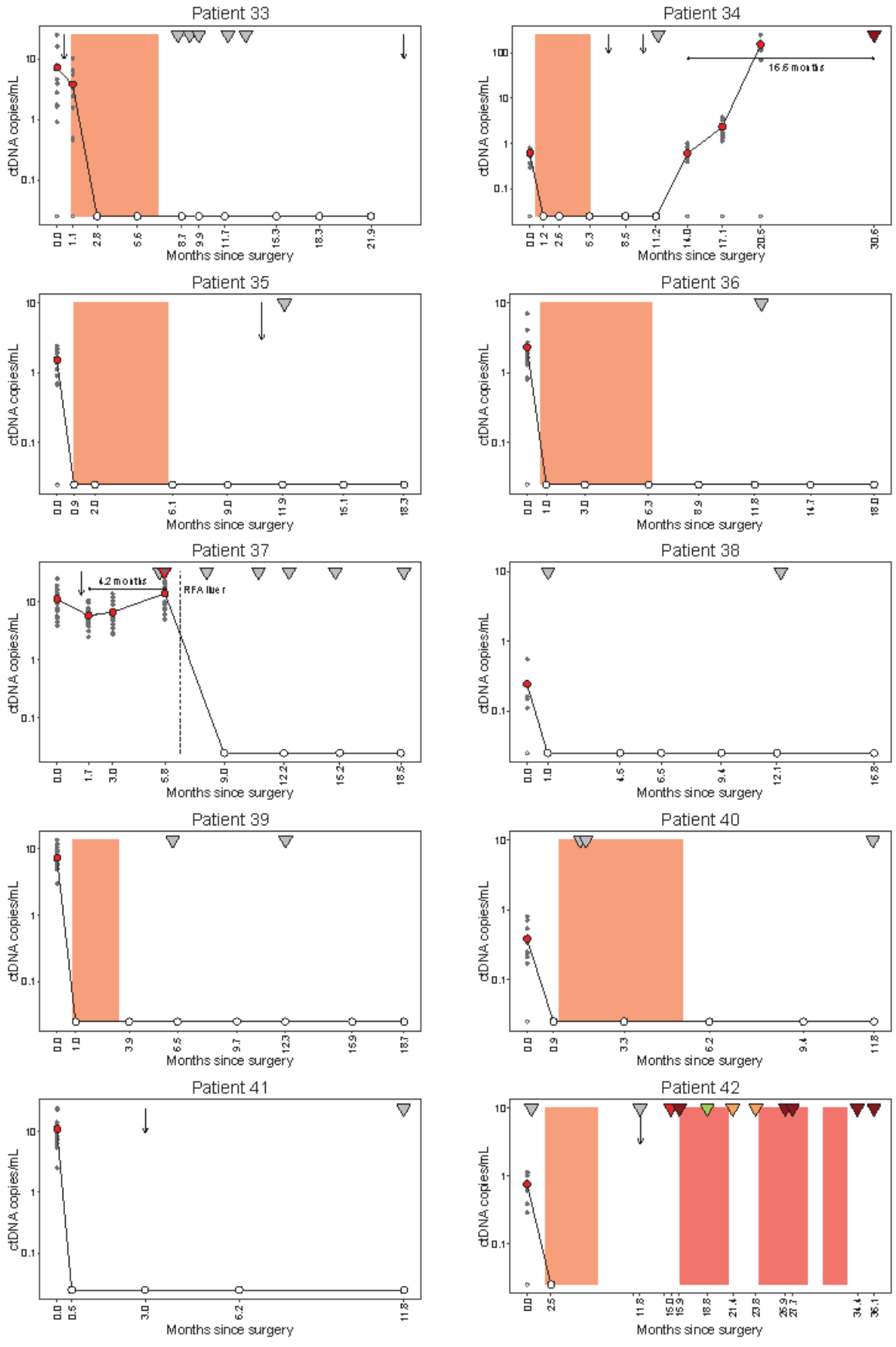


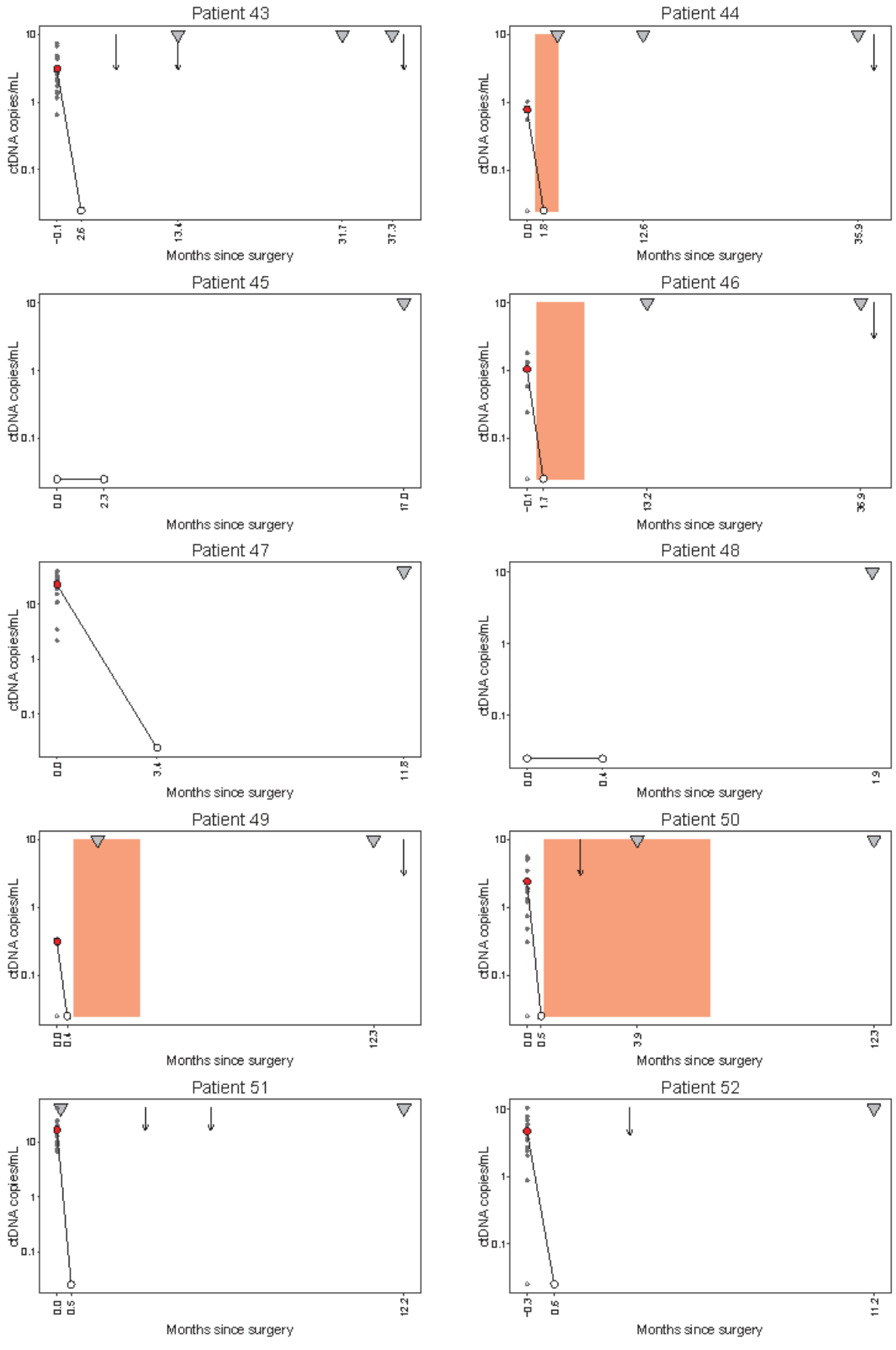


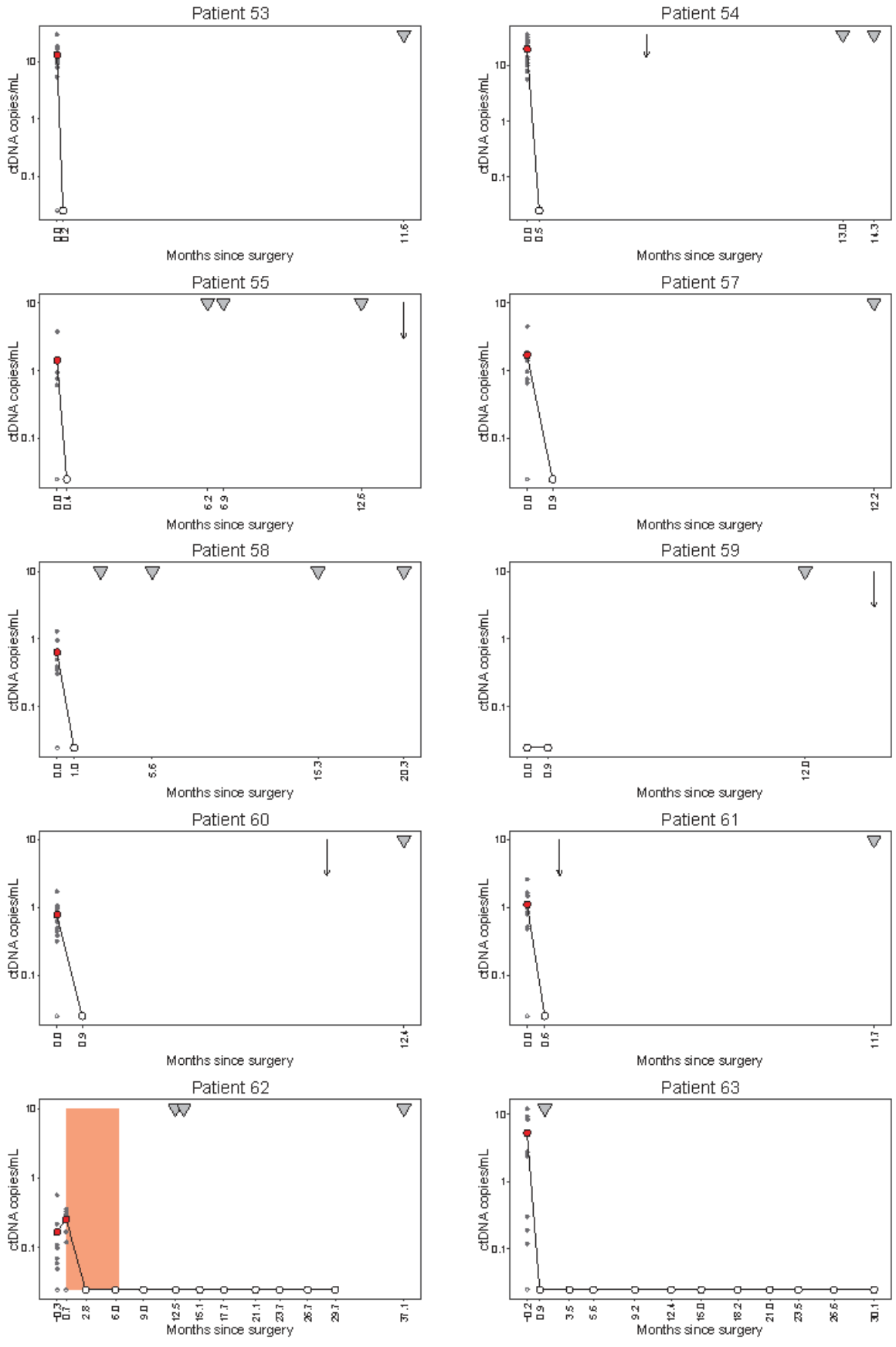


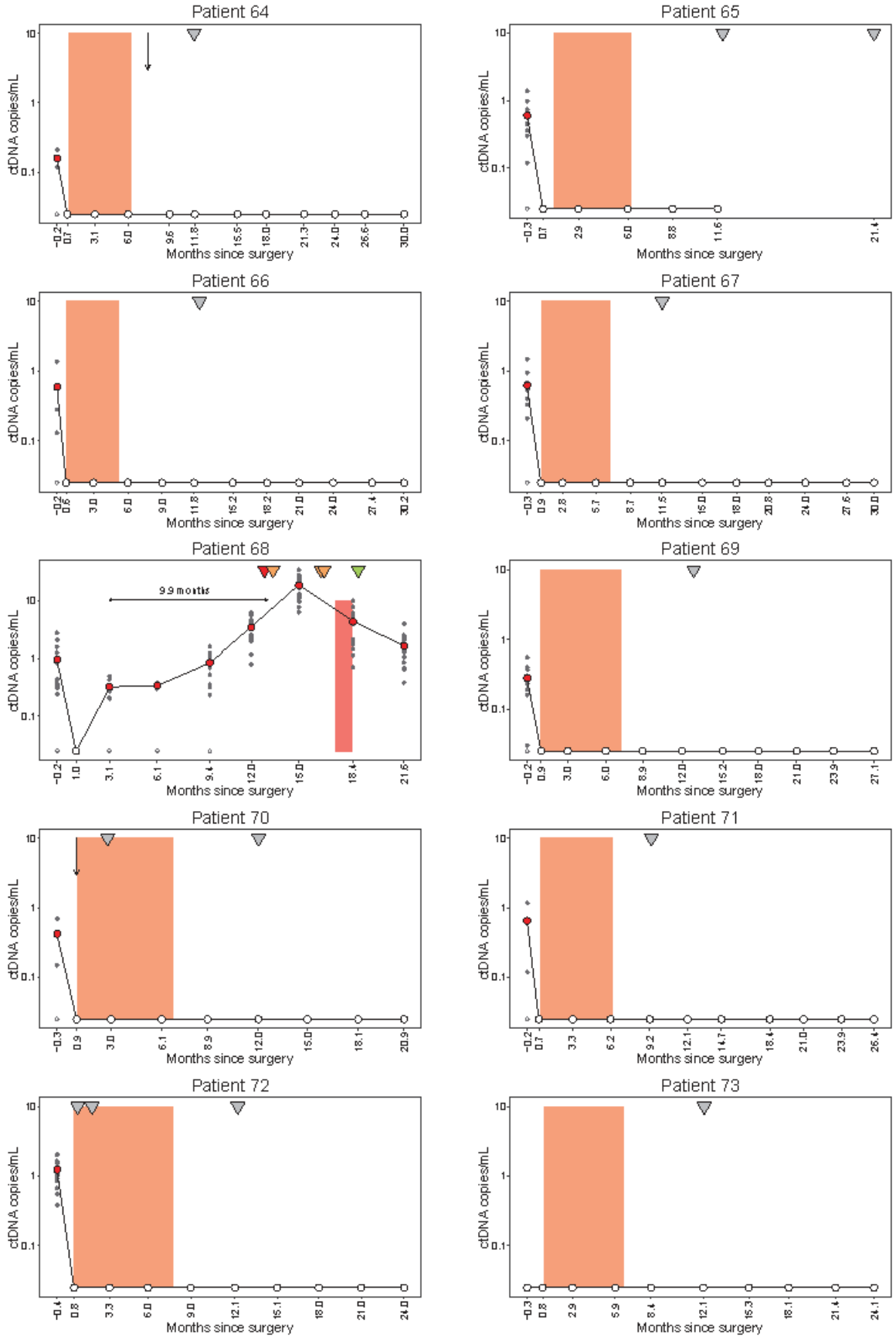


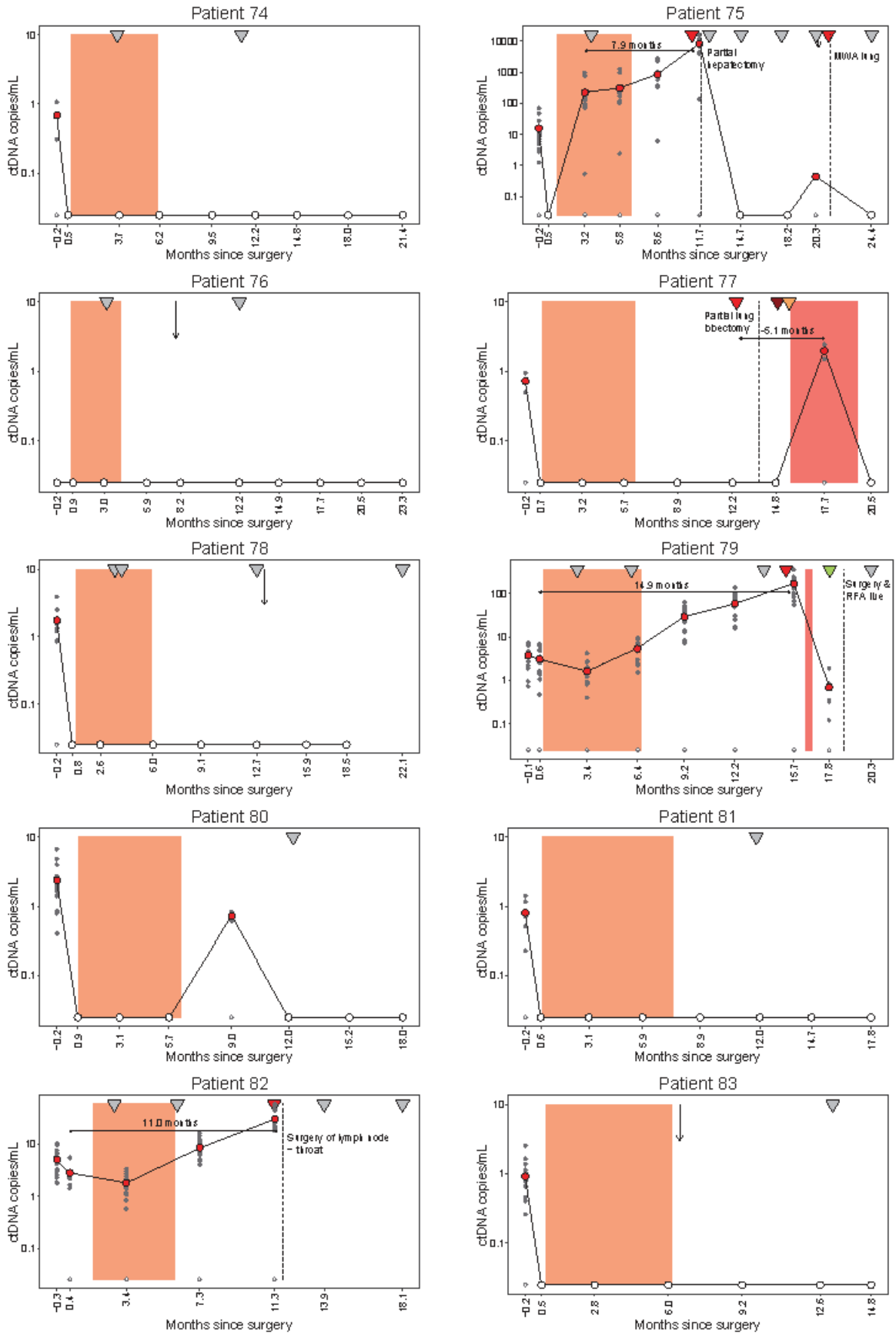


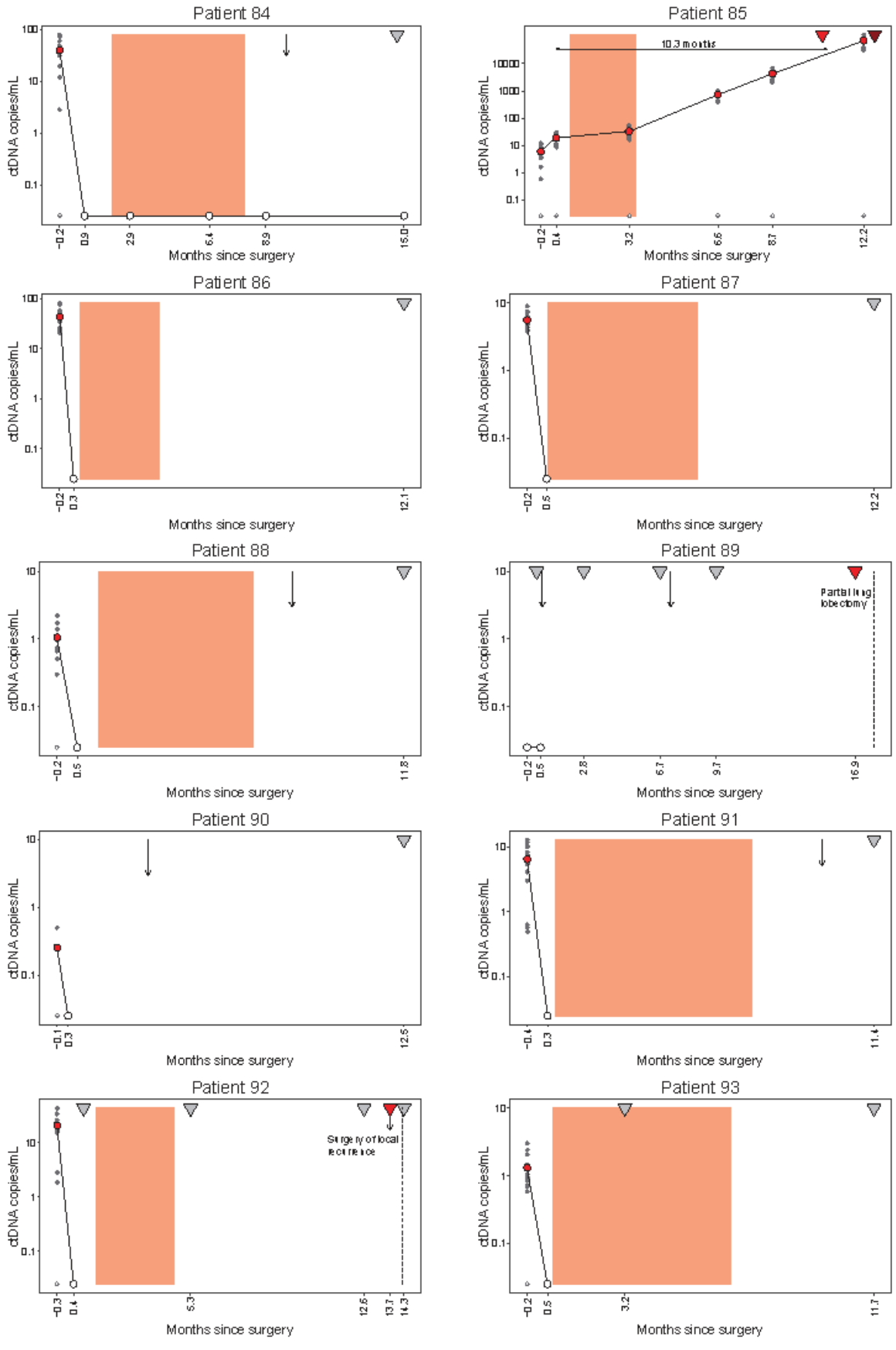


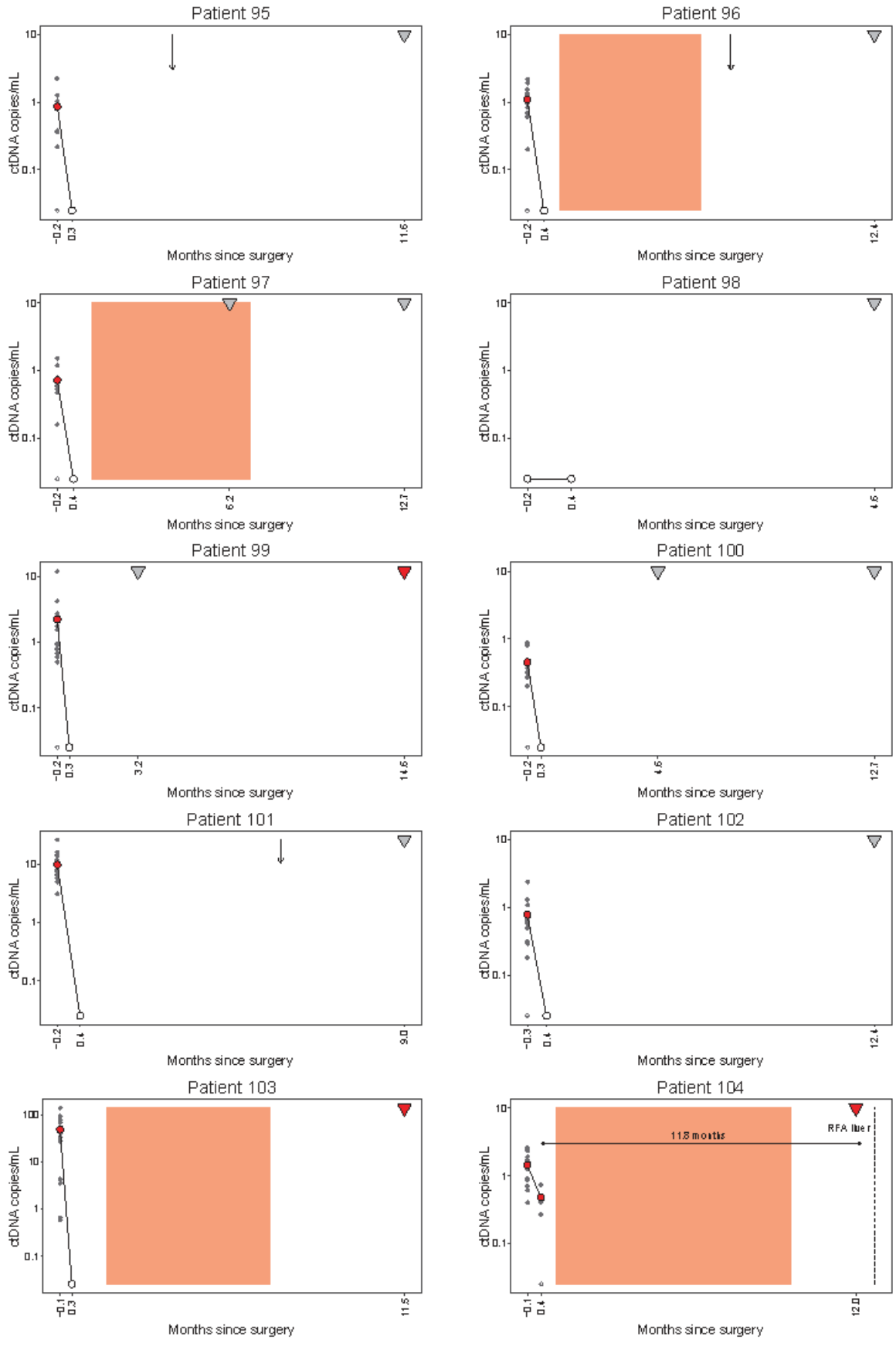


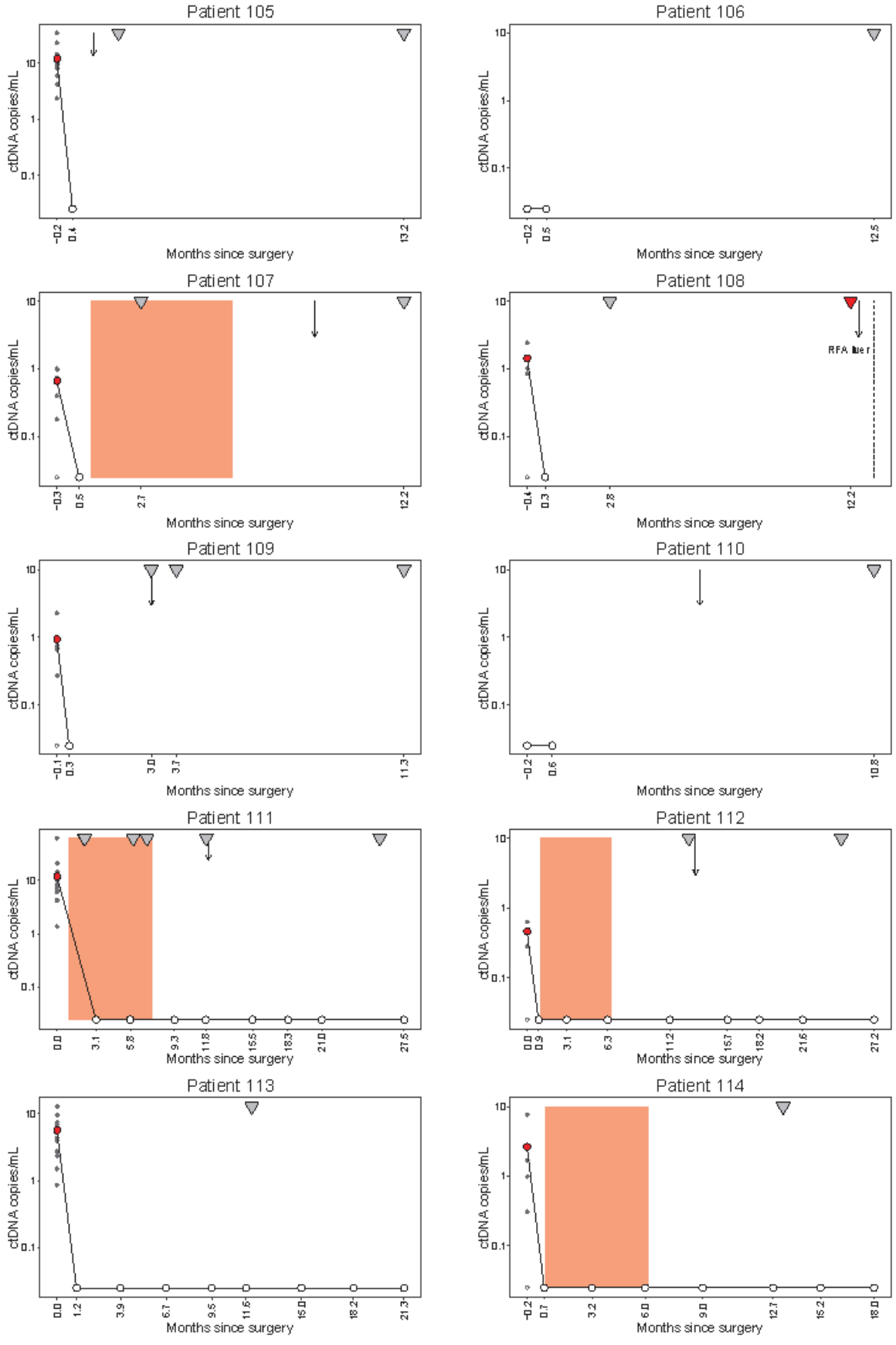


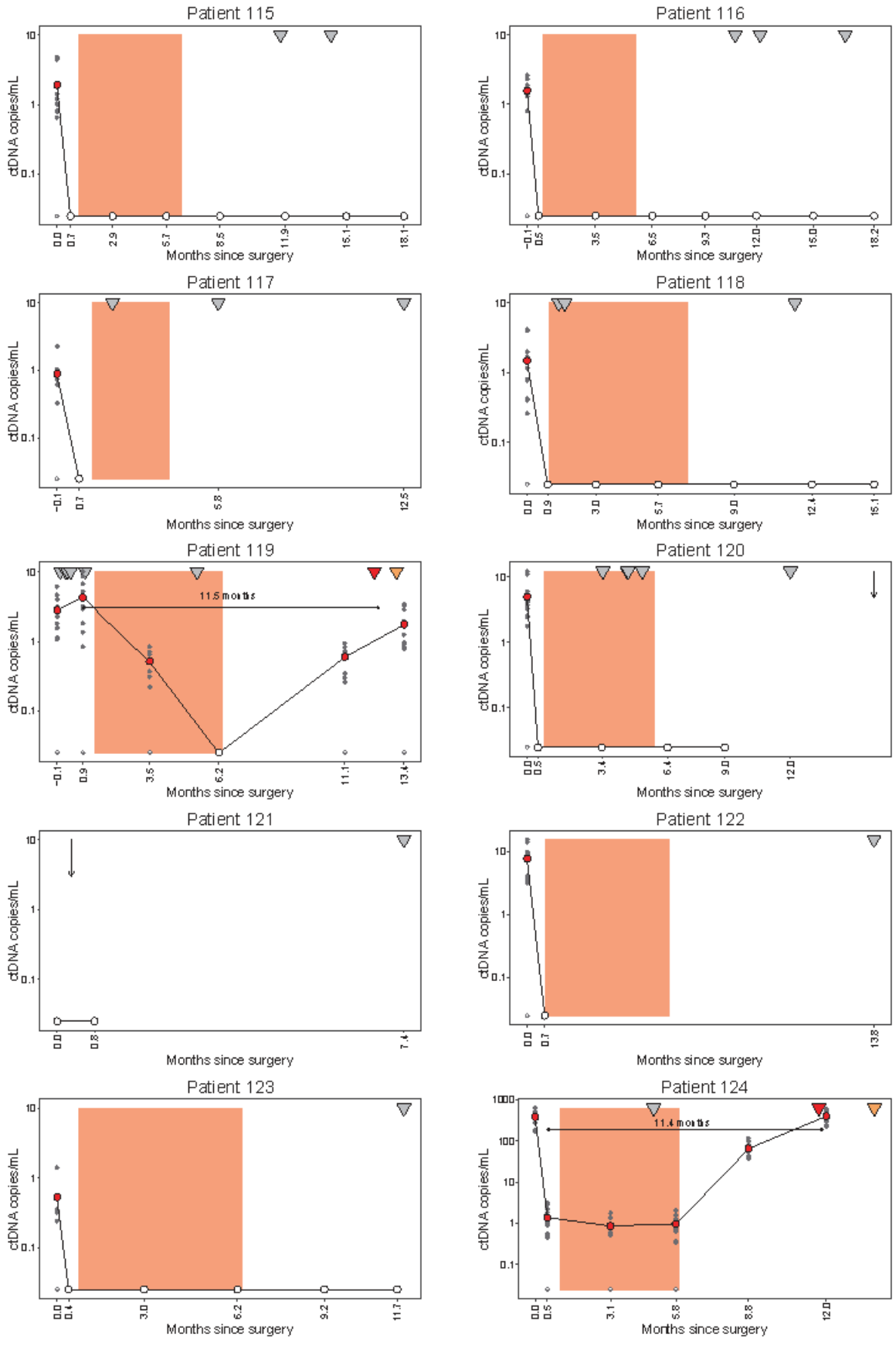


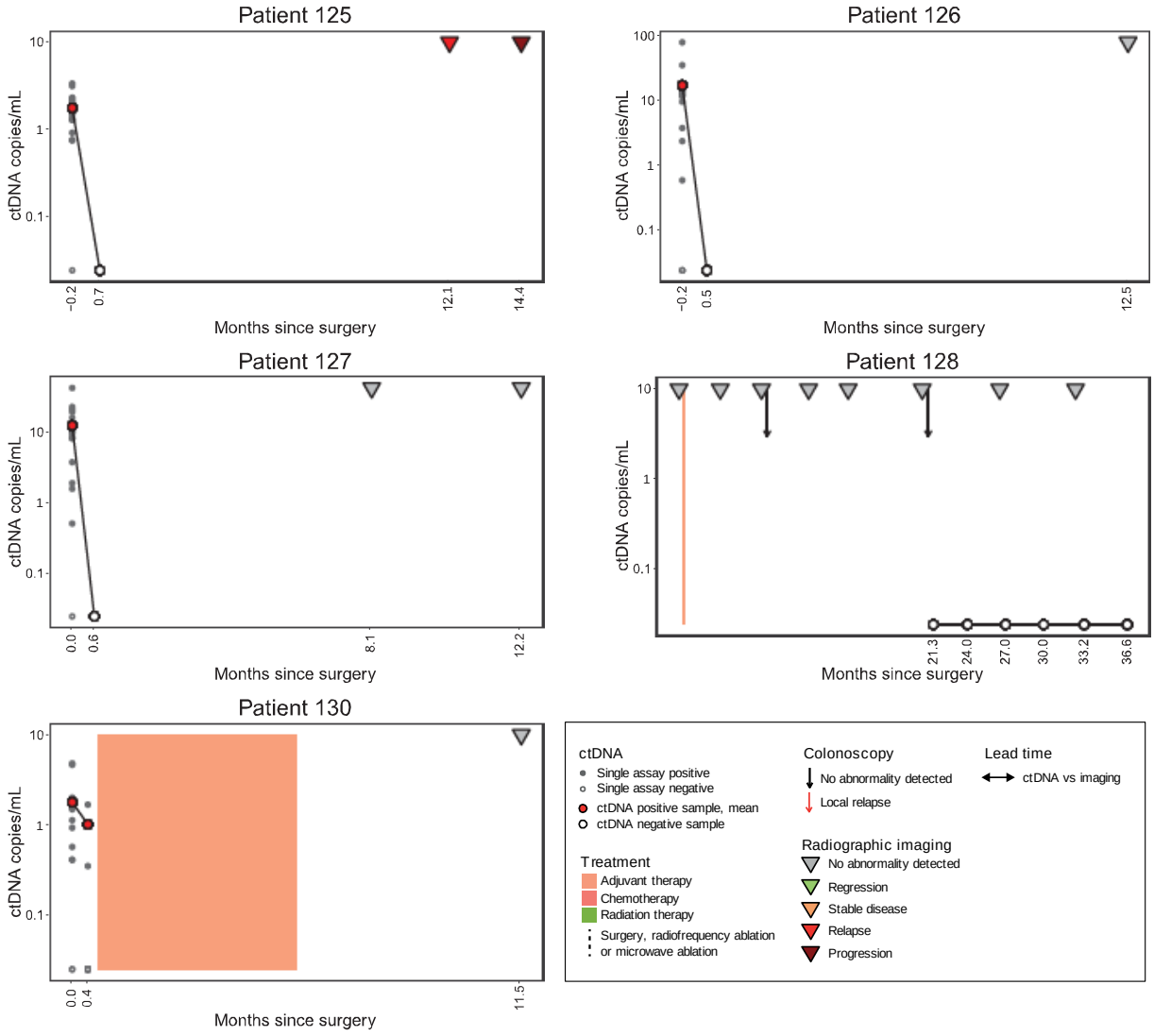




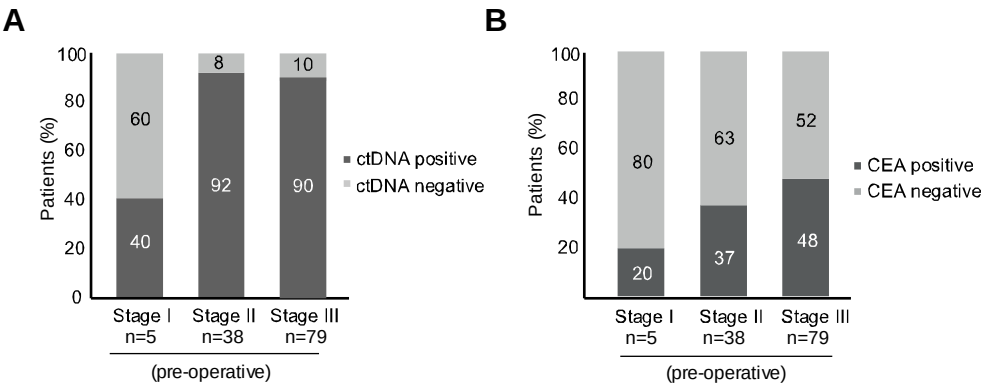




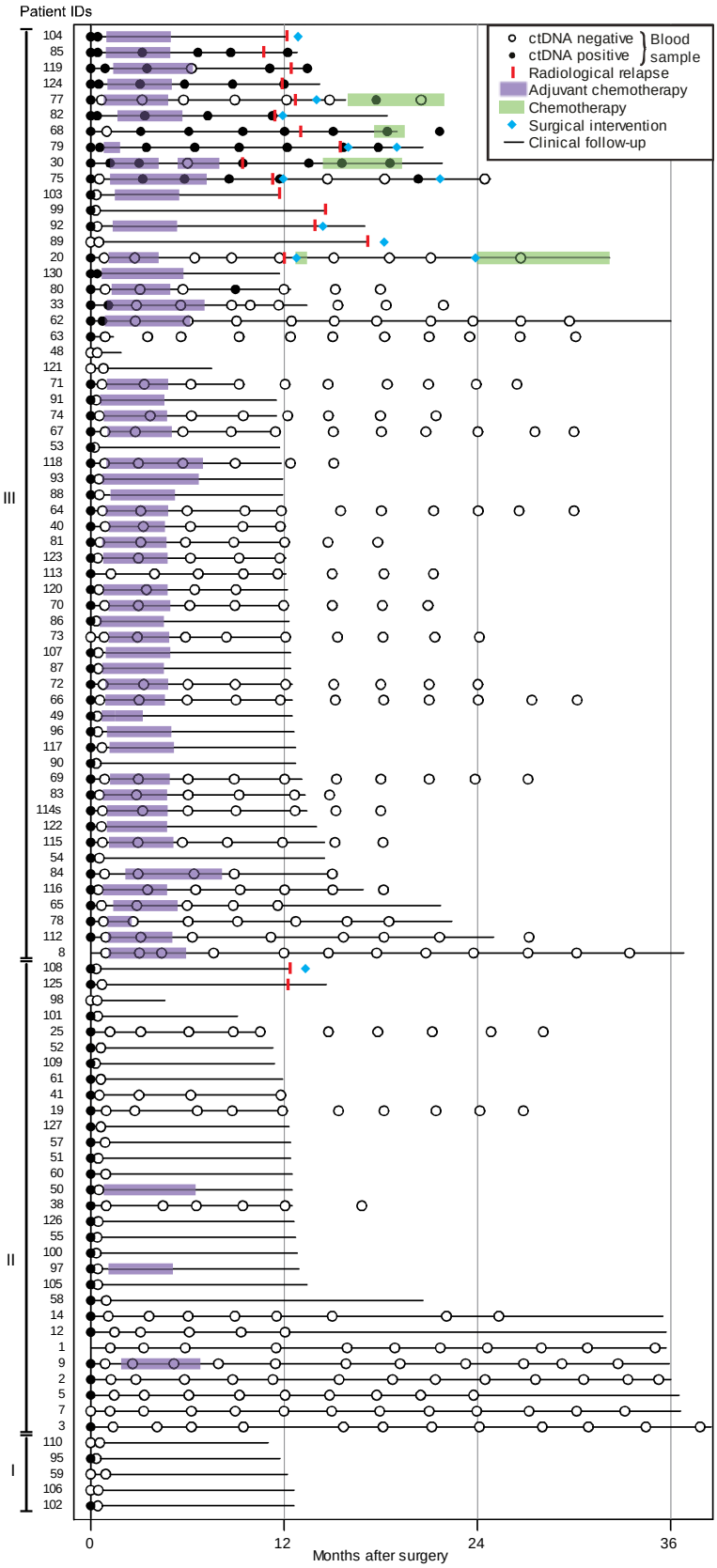




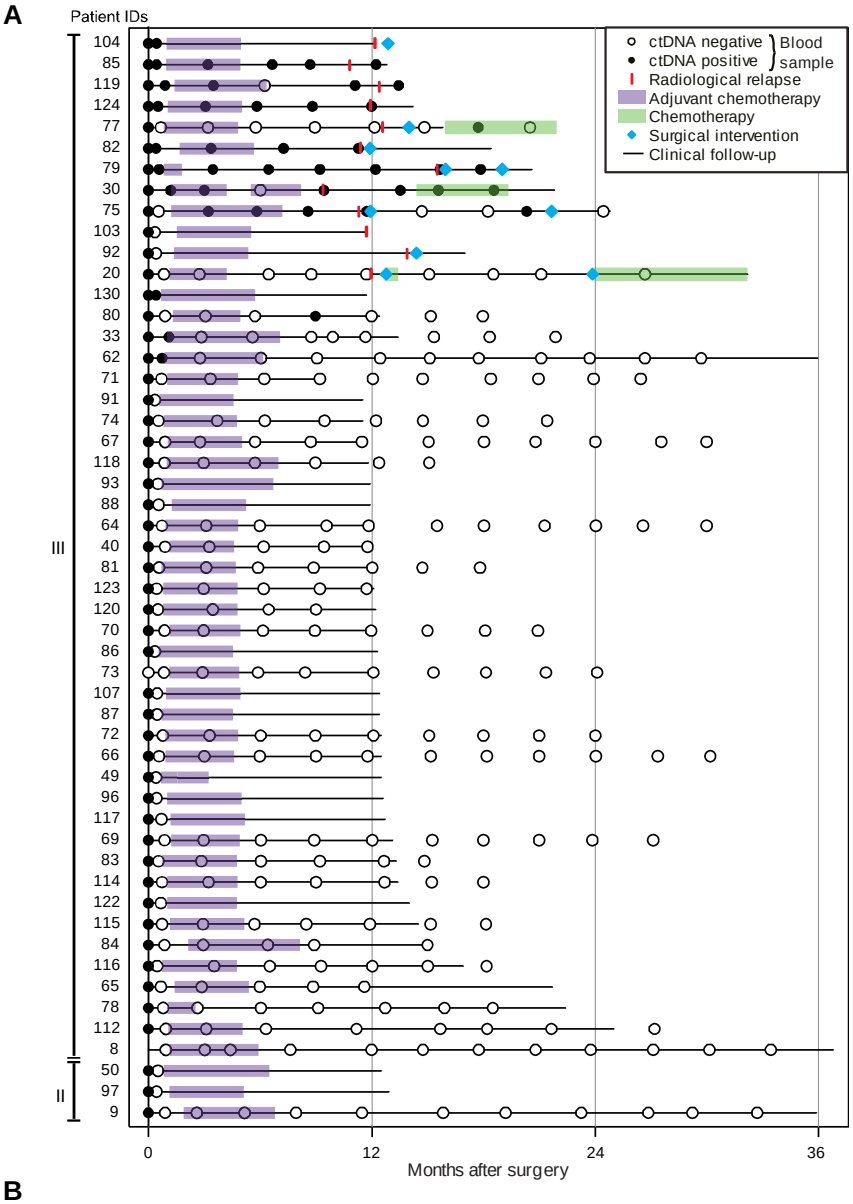
eFigure 4. Pre-operative Detection of ctDNA and CEA in 122 Stage I-III CRC Patients.

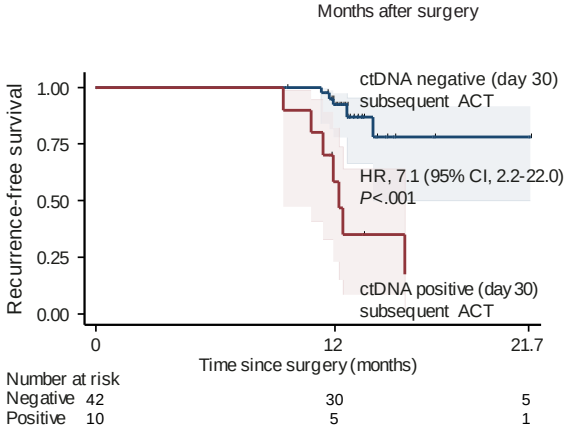


eFigure 5. ctDNA Profiling Results From the 94 Patients Included in the Day 30 ctDNA Analysis. Patients are ordered by recurrence status and disease stage.

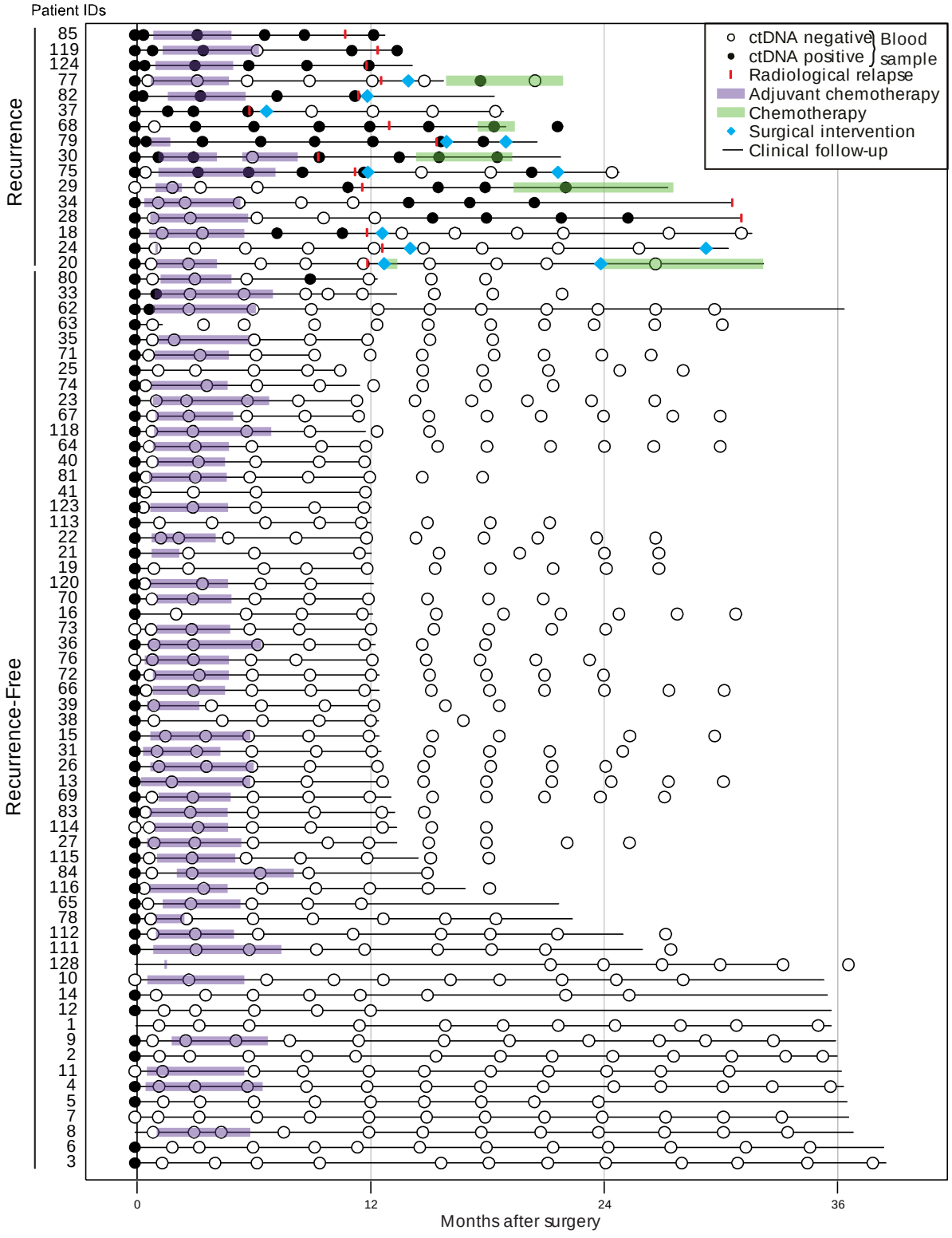


eFigure 6. ctDNA Profiling Results of the ACT Treated Fraction of Patients (n=52) Included in the Day 30 ctDNA Analysis. A) Schematic overview of the ctDNA results. Patients are ordered by recurrence status and disease stage. B) Kaplan-Meier estimates (CI=95%) of recurrence free survival the 52 ACT treated patients, stratified by post-operative day 30 ctDNA status. The Kaplan Meier plot were halted when the proportion of patients in follow-up was under 10%.

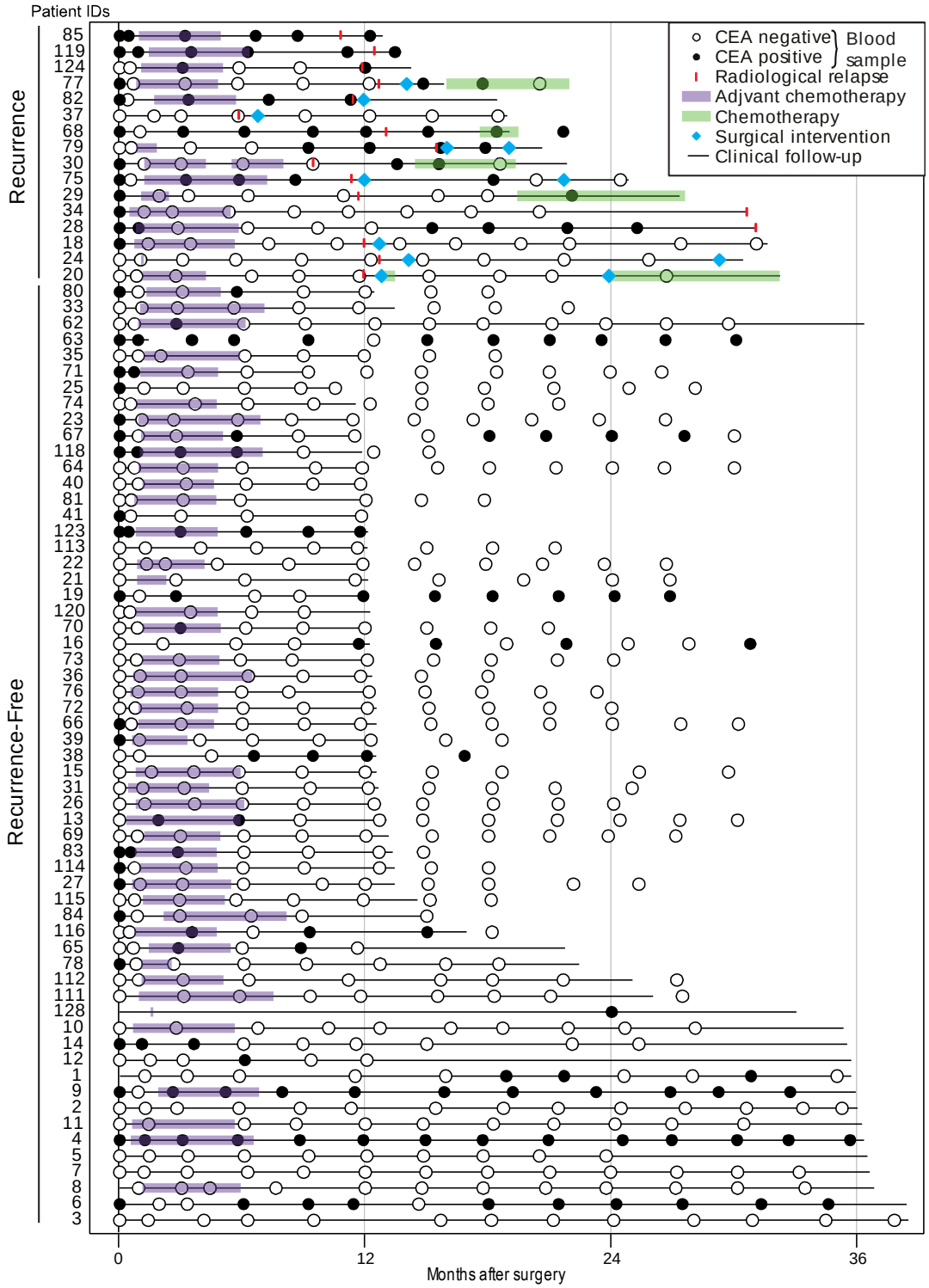




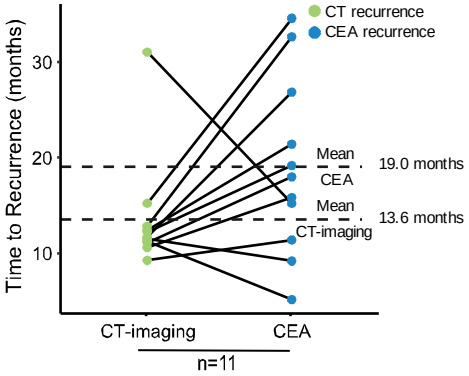
eFigure 7. ctDNA Profiling Results From the 75 Patients Included in the Longitudinal Post-Definitive-Treatment ctDNA Analysis. Patients are ordered by recurrence status. Patients were considered positive if one or more plasma samples post-definitive-treatment was ctDNA-positive. Patient 80, which had a transient ctDNA-positive call at month 9 is likely false positive. From eFigure 3 it can be seen that only 2/16 mutations were called positive at month 9. All other post-operative samples from patient 80 were negative. In all other ctDNA-positive patients positivity persisted in the longitudinal samples and ctDNA status only changed to negative in case of clinical intervention.



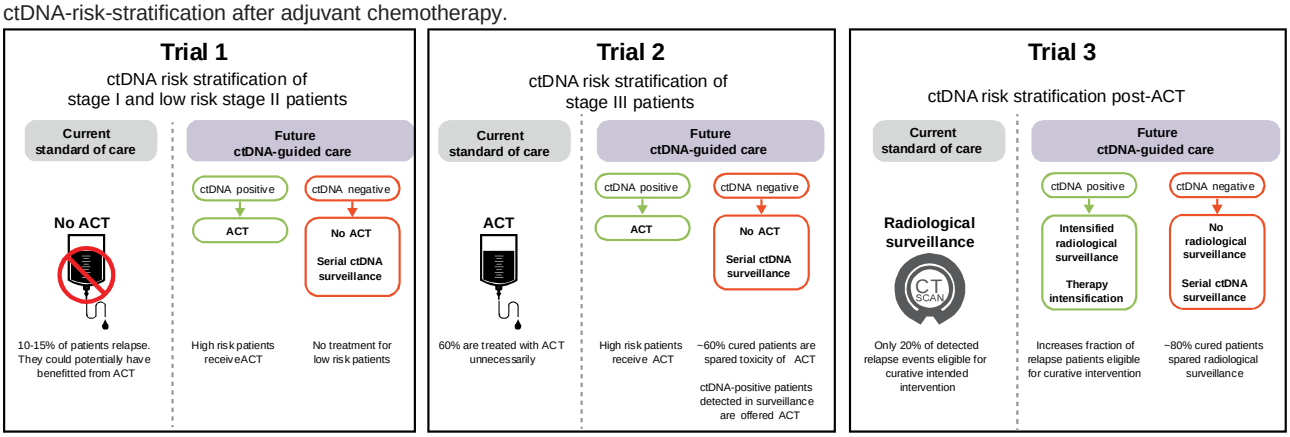
eFigure 8. CEA Profiling Results From the 75 Patients Included in the Post-Definitive Treatment ctDNA Surveillance Analysis. Patients are ordered by recurrence status. Patients were considered positive if one or more surveillance samples were CEA positive.



eFigure 9. Comparison of Time to Recurrence by CEA and Standard-of-Care CT Imaging. The average time from surgery to relapse detection was 19 months for CEA and 13.6 months for CT imaging



eFigure 10. Clinical Trial Proposals for Investigating the Clinical Benefit of ctDNA-Guided Post-Operative Management of CRC Patients. In the present study, we show that detection of circulating tumor DNA post-operatively, defines a patient subgroup with residual disease and very high risk of clinical recurrence, while ctDNA negative patients have low risk of recurrence. Based on these findings we suggest three clinical trials, aimed at investigating the clinical benefit of using ctDNA to guide the post-operative management. Trial 1: here we suggest investigating the effect of offering adjuvant chemotherapy to post-operative ctDNA-positive stage I and low risk stage II patients, who currently would not receive adjuvant chemotherapy. Trial 2: here we suggest investigating the effect of withholding adjuvant chemotherapy from ctDNA negative stage III patients, and hence likely cured patients, with the aim to spare them from unnecessary toxicity. Instead, the patients may be monitored by ctDNA based surveillance. Trial 3: here we suggest investigating the effect of differentiating the intensity of follow-up based on ctDNA-risk-stratification after adjuvant chemotherapy.



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